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SUDDEN CARDIAC DEATH

Second USA-USSR Joint Symposium
Indianapolis, Indiana
December 6-8, 1979

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health

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Office of the Director
National Heart, Lung, and Blood Institute

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health

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PREFACE

The Second USA-USSR Joint Symposium on Sudden Death was held at the Indiana University School of Medicine in Indianapolis, Indiana, on December 6-8, 1979. [The proceedings of the first symposium are published as DHEW Publication No. (NIH) 78-1470.]

The presentations at the second symposium focused on four topics: identification of the patient at risk, cardiac electrophysiology, pathological anatomy and neural activity, and antiarrhythmic drugs. These are the areas in which information is being gathered and exchanged between the two countries and in which scientists are working together on joint research projects of mutual interest.

All of these activities are conducted under the US-USSR exchange in cardiovascular diseases. Sudden death is the fifth problem area among seven areas in this exchange program conducted under the bilateral Agreement in the Field of Medical Science and Public Health between the governments of the United States and the Soviet Union.

At the symposium, 10 American papers and 11 Soviet papers were presented and discussed during the morning scientific sessions. The participants then separated into smaller groups for afternoon workshops in each of the four topic areas. The discussions in these workshops were organized around pertinent displays developed by the staff of the Krannert Institute of Cardiology. The total experience was one of active cooperation in focusing on the overall problem of sudden death. This area of joint activity continues to develop new understanding of the mechanisms responsible for sudden death as well as new approaches for modifying these events. It is hoped that the collection of papers presented at the symposium and contained in these proceedings will stimulate further research on this problem.

The US coordinator for this problem area is Dr. Bernard Lown, professor of cardiology at the Harvard University School of Public Health; Professor Anatoli M. Vikhert, chief of the department of human cardiovascular pathology, National Cardiology Research Center, USSR Academy of Medical Sciences coordinates the USSR program. Dr. Barbara Packard, associate director for cardiology, Division of Heart and Vascular Diseases, is the Institute liaison for this program area.

On behalf of the National Heart, Lung, and Blood Institute, I wish to thank the US and USSR chairmen, other US and USSR participants, and all those in both the USSR and USA who participated in the planning and conduct of the symposium and editing of the proceedings. Very special thanks go to Dr. Douglas P. Zipes and his colleagues and staff at the Indiana University School of Medicine and Krannert Institute of Cardiology for their warm hospitality and painstaking efforts to help make the symposium a success.

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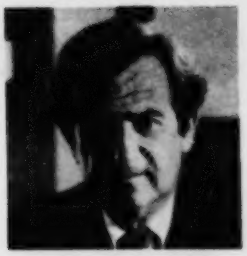

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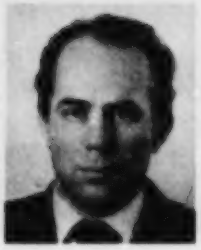

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PART I

IDENTIFICATION OF THE PATIENT AT RISK

PREVALENCE OF CARDIAC RHYTHM DISTURBANCES IN A POPULATION SAMPLE

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ABSTRACT. An 0.7 percent sample (164 men) of the 35- to 59-year-old male population in one of the Moscow areas was examined to determine the frequency and nature of cardiac rhythm disturbances. The examination methods were: questions using a special chart, including the Rose questionnaire; physical examination; electrocardiogram (ECG) at rest; 24-hour monitored ECG; measurement of arterial pressure; determination of biochemical indices (cholesterol level, blood sugar, and others); determination of heart size (by roentgenography); and anthropometry. The examination included 139 men (84.8 percent of the total), 110 (or 79.1 percent) of whom were included in the 24-hour ECG monitoring program. Seventeen of the 139 men (12.2 percent) were found to have ischemic heart disease (IHD). Ventricular or supraventricular rhythm disturbances during the 24-hour monitoring were found in 77 percent: 65 percent had ventricular extrasystoles, 54 percent had supraventricular disturbances, and 42 percent had both ventricular and supraventricular extrasystoles. Evaluation of ventricular extrasystoles by maximum grades among those without signs of IHD showed that 28 percent were grade 1, 21 percent were grade 3, and 12 percent were grades 4-5. In persons with IHD, ventricular extrasystoles were distributed as follows: 14 percent were grade 1, 43 percent were grade 3, and 29 percent were grades 4-5. Grade 3 and higher ventricular extrasystoles were statistically significant more often ($p < 0.05$) in persons with IHD than in persons without IHD.

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INTRODUCTION

A number of papers have reported on the frequency and nature of rhythm disturbances found by 24-hour ambulatory monitoring of selected groups (1-4). The purpose of the present work was to study the prevalence of arrhythmias among a sample population.

MATERIALS AND METHODS

An 0.7 percent sample (164 men) was randomly selected from 23,157 males aged 35 to 59 years and living in one of the areas of Moscow. Of these, 139 (or 85 percent) were examined. Monitored ECG study was conducted in 123 of the total 164 (75 percent), but only 110 of the 164 (67 percent) were subjected to 24-hour ambulatory monitoring. Examination, in addition to the monitoring, included standard questioning to elucidate angina pectoris of effort, intermittent claudication and smoking habits, a general physical examination of the patient, measurement of arterial pressure, ECG at rest, cholesterol level, fasting blood glucose and glucose 1 hour after a 50 g glucose load (per os), roentgenographic determination of heart size, and anthropometry. Definite chronic IHD was established based on one of the following criteria:

- Typical angina of effort
- Presence on the ECG of signs of previous myocardial infarction: (1-1, 1-2 on the Minnesota code), or anamnestic data showing previous evidence of myocardial infarction
- Presence on the ECG of signs of chronic coronary insufficiency: 5-1.2 and 4-1.2 (without 3-1), as well as 7-1, 6-1, and 8-3 (lacking other reasons for atrial flutter).

Cases in which the angina pain syndrome was not completely typical, or in which changes of the type 1-3, 4-3, 4-4, and 5-3 were found on the ECG, were included in the group with possible IHD.

Arterial hypertension was diagnosed in cases where arterial pressure was $\geq$ 160/95 mm Hg, carbohydrate tolerance was impaired, and fasting glucose was $\geq$ 91 mg% and $\geq$ 151 mg% 1 hour after receiving 50 g glucose. Smoking was regarded as a risk factor if the patient smoked 10 or more cigarettes a day. Obesity was recorded if the patient's relative weight was 15 percent or more in excess of the norm, as determined from Davenport's tables.

The 24-hour ECG monitoring of the patients under conditions of normal activity was carried out by a two-channel recorder manufactured by Avionics Corporation (USA); this was followed by an independent analysis of the ECG by two physicians. Each new type of arrhythmia was recorded on a paper tape. In the event of different conclusions reached by the physicians, final diagnosis was made with a third specialist. The grades proposed by Lown and Wolf were used to analyze ventricular extrasystoles. Heart volume was established by roentgenography, and size was calculated using the method described by Rabkin et al. (5). The roentgenograms were evaluated independently by two specialists.

Disagreements were judged by a third arbitrator. The difference between the estimates of heart volume by the two investigators was slight and amounted to 1.2 percent of the mean value of the heart volume (M_1 = 726.3 cc, M_2 = 717.6 cc). The value taken for analysis represented the mean of the two estimates. Roentgenologic examinations were performed on 97 subjects.

RESULTS

Ischemic heart disease was found in 12.2 percent of those examined; arterial hypertension was found in 33 percent, blood serum cholesterol > 220 mg% in 33 percent, carbohydrate tolerance disturbance in 52 percent, excessive smoking in 44 percent, and obesity in 23 percent.

Rhythm disturbances (supraventricular and ventricular extrasystoles) were found during 12-lead ECG recordings in 0.9 percent of the cases, and in most cases (77 percent) during 24-hour recordings (table 1).

Ventricular extrasystoles occurred most often (in 65 percent) among those examined. Ventricular extrasystoles occurred in a majority (42 percent) of those with supraventricular extrasystoles (54 percent). Through analysis of the circadian cycle we noted persistent sinus tachycardia (in excess of 100 beats per minute over a minimum period of 1 hour) in 14 percent of the cases. Group supraventricular extrasystoles were recorded in 8 percent of those examined; only 2 percent had short episodes of supraventricular tachycardia.

Ventricular tachycardia was also short-lived, consisting of three to five complexes in two cases and of more than five ventricular complexes in another two cases. One person was found to have had a short episode of accelerated idioventricular rhythm. Only one person was found to have the parasystole type of ventricular extrasystole. Polytopic ventricular extrasystoles with three and more foci were recorded in 21 percent.

Table 2 gives the results of analysis of ventricular extrasystoles by maximum grades. No disturbances in ventricular rhythm were found in one-third

TABLE 1. Frequency of Cardiac Rhythm Disturbances
Found by 24-Hour ECG Monitoring of 35- to 59-Year-Old Males

Type of disturbance	Percent of patients
Ventricular or supraventricular extrasystoles	77
Ventricular extrasystoles	65
Supraventricular extrasystoles	54
Ventricular and supraventricular extrasystoles	42

TABLE 2. Frequency of Maximum Grades of Ventricular Extrasystoles (VE) Among a Male Random Sample

VE grade	VE frequency (%)		
	Among all males examined	Among IHD patients	Among patients without IHD
0	35	14	39
1	26	14	28
2	-	-	-
3	24	43	21
4 + 5	15	29	12

IHD = Ischemic heart disease.

(35 percent) of all men examined, whereas more than one-third (39 percent) were found to have ventricular extrasystoles of grade 3 and higher. Occasional monotopic extrasystoles were recorded in one-fourth (26 percent) of those examined.

While it is true that the number of patients with chronic IHD is small, the differences in the frequency of complex grades (3 and higher) are statistically significant ($p < 0.01$). The recordings for patients without IHD showed less frequent ventricular extrasystoles than was the case among those with IHD.

Table 3 provides data which characterize in general the frequency of various grades of extrasystoles for 1 hour out of every 24 for men examined by 24-hour ECG recordings and by analysis of rhythm disturbance based on maximum grade.

The greatest difference between patients suffering from IHD and those without IHD was noted in the frequency of grade 2 ventricular extrasystoles: The frequency was greater among those with IHD by a factor of 6. Statistically significant differences also were found in other groups. Monotopic ventricular extrasystoles and grades 4-5 extrasystoles occur among patients with possible IHD, despite the small number of patients observed, much more frequently than they do among patients without IHD.

We did not intend to study the relationship between rhythm disturbances and IHD risk factors because of the small random sample used. But, we did establish the frequency distribution of various cardiac volumes among the examined population in order to determine the relationship between heart

TABLE 3. Frequency of Various Grades of Ventricular Extrasystoles (VE) In 35- to 59-Year-Old Males (in %)

Patient group	Patient characteristic	VE grade				SVE
		1	2	3	4-5	
1	IHD patients	85.7**	28.6**	64.3**	28.6*	57.1
2	Possible IHD patients	83.3*	-	33.3	33.3*	83.3
3	Patients without IHD	60.0	4.4	30.0	12.2	51.1
TOTAL	110 men	71	8	38	17	

*p < 0.05 compared with group 3
**p < 0.01 compared with group 3
SVE = Supraventricular extrasystole. IHD = Ischemic heart disease.

rhythm disturbance and cardiac volume (figure 1). According to some data (5), the upper limit of the norm is 720 cc. This figure corresponds to the limit between the second and third quartiles on our distribution histogram. In other words, some 50 percent of the population examined had "increased" heart size, which is very unlikely. This led to an analysis in which we established heart sizes for patients with IHD, for patients with arterial hypertension (AH), and for healthy persons (i.e., persons without IHD, AH, rheumatic or other heart disease, and without severe damage to other organs). We found that the mean volume among essentially healthy persons was 705 ± 16 cc, and that the mean volume for patients with IHD or AH was higher, or 754 ± 23 cc. The difference was not statistically significant (t = 1.80, p > 0.05). The distribution of heart volumes for essentially healthy persons showed that the 80 percent percentile was between 800-810 cc and that the 90 percent percentile was between 880-890 cc.

Analysis of data on the relationship of cardiac volume and ventricular extrasystole established that persons without IHD and without a ventricular extrasystole have the smallest mean volume (710 ± 18 cc), whereas the maximum heart volume was found in IHD patients with a ventricular extrasystole (779 ± 46 cc). The group of patients without IHD but with a ventricular extrasystole had a mean heart volume intermediate between the other two groups (table 4).

Table 5 gives the characteristics of ventricular rhythm disturbances by maximum grades among healthy people. Ventricular extrasystoles were seen in 63 percent of those examined in this group.

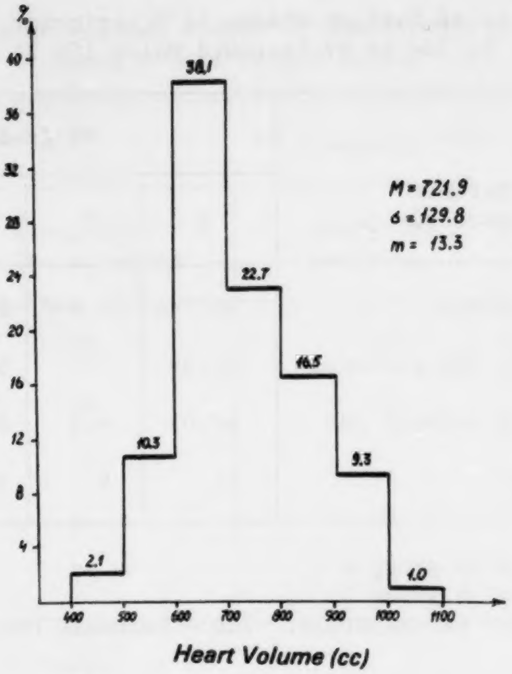

FIGURE 1. Histogram of distribution of heart volumes in a sample of 35- to 59-year-old males.

TABLE 4. Mean Cardiac Volumes Among Various Groups of Patients Examined

Group examined	No. examined*	Volume (M ± m) (cc)
1. Persons with IHD and VE	9	779.0 ± 46.1
2. Persons without IHD and without VE	29	710.3 ± 18.1
3. Persons with VE and without IHD	54	736.6 ± 17.8
4. Persons with IHD and without VE	2	605.0 ± 15.0

*The study covered 94 of the 97 persons on whom heart measurements were taken. IHD = Ischemic heart disease. VE = Ventricular extrasystole.

TABLE 5. Frequency of Ventricular Extrasystoles (VE) by Maximum Grades Among Healthy Persons

VE grade	Frequency (%)
0	37
1	27
2	–
3	20
4a	2
4b	3
5	11

Complex grades of ventricular extrasystoles (grade 3 and higher) were observed in one-third (36 percent). Early extrasystoles (prematurity index less than 1) were recorded among 11 percent.

The total number of ventricular extrasystoles was not more than 10 among 70 percent of the essentially healthy persons with ventricular extrasystoles. Only 20 percent were found to have more than 20 per day.

Persons with cardiac volumes greater than 810 cc were found one and one-half times more frequently (25.4 percent) among those examined and found to have VE during the monitoring study than among persons without VE (15.6 percent) ($p > 0.05$). At the same time, persons with cardiac volumes greater than 880-890 cc (90 percent percentile) were found almost three times as often among persons with VE (17.5 percent) than among persons without VE (6.3 percent) ($p > 0.05$).

The overwhelming majority of those examined (89 percent) were unaware of any disturbance in heart rhythm.

CONCLUSIONS

This first study of a random sample of 35- to 59-year-old males enabled us to determine the frequency distribution of ventricular extrasystoles using 24-hour ECG recordings. However, it is difficult to compare our data with that obtained by other researchers because their data were obtained from selected groups.

Our results confirm that ventricular extrasystoles are widespread among so-called healthy persons, as well as among patients with IHD. These data also indicate that ventricular rhythm disturbances in the population are most often associated with chronic IHD, as well as with increased heart volumes.

A broader epidemiological study is needed to determine more accurately which of the indices of heart volume can be considered conventionally normal. Appearance of ventricular extrasystoles in a healthy person will thus demand more extensive examination because such appearance is often found with comparatively increased heart size.

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REDUCTION OF VENTRICULAR ECTOPIC ACTIVITY BY HYDRALAZINE IN SURVIVORS OF OUT-OF-HOSPITAL VENTRICULAR FIBRILLATION: A SINGLE-BLIND, PLACEBO-CONTROLLED TRIAL

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ABSTRACT. We tested the hypothesis that improvement in left ventricular function might result in a reduction of ventricular premature beats (VPB's). Ten ambulatory survivors of out-of-hospital ventricular fibrillation were admitted to the hospital for these studies. Patients were selected on the basis of having frequent ventricular ectopics (more than 60 per hour) plus findings of left ventricular dysfunction (cardiac enlargement and/or reduced ejection fraction). All patients had coronary heart disease. The patients received two doses of the vasodilator, hydralazine, on 1 day and two placebo doses on the other day. The medications were given in a single-blind randomized protocol. Each of the 10 subjects served as his own control, using 10 hours of basal electrocardiographic monitoring on both hydralazine and placebo days. The change in ventricular ectopy from pretreatment basal levels was significantly reduced with hydralazine compared to the placebo. The average change in ventricular ectopy was +28 VPB's per hour with the placebo compared to -5 VPB's per hour with hydralazine ($p < 0.002$). Although blood pressure, heart rate, and pulmonary "capillary" wedge pressures did not change significantly, gated blood pool radionuclide ventriculography showed a significant reduction in both right and left ventricular end-diastolic and end-systolic counts. Hydralazine significantly reduced heart size and

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ventricular ectopic activity in 10 survivors of the sudden cardiac death syndrome. It is probable that the effect on the VPB rate was a consequence of altered ventricular function from hydralazine. Further controlled trials in larger numbers of patients seem warranted.

INTRODUCTION

There is little doubt that one of the major problems confronting modern cardiology relates to the prevention of sudden cardiac death. Lown and colleagues have presented studies suggesting that chronic suppression of complex VPB's may prevent the emergence of ventricular fibrillation and sudden cardiac death (1). This remains an unsettled issue. Ventricular premature beats, particularly the so-called complex VPB's, are known to be associated with the development of sudden cardiac death in the months following acute myocardial infarction (2,3). A similar relationship has been noted in patients who have previously been resuscitated from out-of-hospital ventricular fibrillation (4). Other predictors of sudden cardiac death include left ventricular dysfunction manifested by congestive heart failure (5) and the extent of coronary artery obstruction (6). There are data suggesting that ventricular ectopic activity and impaired ventricular function may be independent predictors of sudden death (2,7). However, this independence has not been clearly established. In experimental preparations myocardial fiber stretch has been shown to be arrhythmogenic (8,9).

The purpose of our study was to determine whether improvement in left ventricular function would result in a demonstrable reduction of ventricular ectopy. To test this relationship, we administered a vasodilator agent, hydralazine (10,11), to survivors of out-of-hospital ventricular fibrillation who had coronary heart disease with impaired left ventricular dysfunction and frequent VPB's.

METHODS

Patient Selection

Several years ago we established a research clinic to follow up patients who had been resuscitated from out-of-hospital ventricular fibrillation by the Seattle Fire Department's emergency care system. About 100 such patients are enrolled each year. We reviewed the records of ambulatory patients attending this clinic to select a group of persons with coronary heart disease who had abnormal ventricular function, and frequent ventricular ectopic activity. Coronary heart disease was assessed by history of myocardial infarction, characteristic electrocardiographic Q waves, coronary arteriography, or combinations of these techniques. Ventricular ectopy was documented by 24-hour continuous electrocardiographic recordings. Patients showing 60 VPB's per hour were considered candidates for study. Left ventricular dysfunction was documented by cardiomegaly on chest X-ray or by reduced ejection fraction determined by contrast or radionuclide left ventriculography. The average age of the 10 patients studied was 65 years. Seven were classified as functional class II and three as class III (classification of the New York Heart Association).

Protocol

The subjects were admitted to the hospital coronary care unit on the day prior to study. Antiarrhythmic medications were discontinued 24 hours prior to study except for propranolol, which was continued in four patients. Regular doses of diuretics and digitalis were administered to three patients in the evenings preceding the days when hydralazine and a placebo were administered.

Continuous electrocardiographic recording was begun in the evening prior to study and continued throughout the next day. On the morning of the first study day, a #7 French, flow-directed, balloon tipped, thermodilution catheter was placed in the pulmonary artery of five patients for monitoring of cardiac output, pulmonary artery, and pulmonary "capillary" wedge pressures. In these and the other five patients standard blood pressure and heart rate determinations were also obtained at hourly intervals.

After the basal period, which averaged nearly 10 hours, an oral dose of either 25 mg hydralazine or a placebo was given in the morning. This was followed 4 to 5 hours later by a second dose of the same preparation: either hydralazine (average 33 mg) or a placebo. On the next day, two doses of the alternate preparation were given at the same intervals. The order of administration was reversed in sequential patients. A gated radionuclide ventriculogram using 25 mCi of technetium-99m-pyrophosphate labeled red blood cells (12) was performed on both study days. These studies were acquired into a dedicated minicomputer. The cardiac cycle was divided into 14 time frames beginning with the R wave. Typically, 250,000 counts per frame were acquired. The heart was imaged in the anterior view and the left anterior oblique (LAO) view which best demonstrated ventricular separation (typically 45° LAO). The basal measurements were acquired just prior to administration of hydralazine or placebo and repeated 2 and 5 hours later. A 10 cc syringe containing blood withdrawn prior to each radionuclide angiogram was attached to the surface of the collimator and surrounded by a lead shield. The activity of this calibrating syringe was measured by simultaneous acquisition with the radionuclide angiogram.

Data Analysis

The data were processed without knowledge of the order in which hydralazine or the placebo was given. The electrocardiographic recordings were scanned by an experienced operator, first using a manual scanning system and subsequently using an instrument designed to detect and count ventricular ectopic activity by analog circuitry (Avionics Model 660). The presence of complex ventricular ectopy was always verified manually.

The count-generated volume data were determined from the LAO view. Each radionuclide ventriculogram was processed by two independent observers and the results were averaged. The counts in the calibrating syringe were determined from a computer-generated area of interest and expressed as counts per ml blood. The images were then "smoothed" by a linear low-band pass noise filter (13). Both right and left ventricles were analyzed. The computer generated a varying region of interest which tracked the decreasing and expanding ventricular size during each of the 14 time frames of the cardiac cycle. End diastole and end systole were identifed by the maximal and minimal counts, respectively. The end-diastolic and end-systolic counts were divided by the counts per ml from the calibrating syringe, resulting in a volume index normalized for initial radionuclide dose and decay. The two treatment values were averaged and compared to the respective basal measurements.

RESULTS

On the first study day five patients received hydralazine and five received a placebo. The basal recording was obtained during the entire 9 to 10 hours prior to administration of the study medication, which was given at about 9:30 a.m. The average of the hourly rates of VPB's during the basal and the study periods is shown in table 1, and the changes in VPB frequency are compared in figure 1. Overall, the change in the hourly VPB rate from the basal to the hydralazine period was significantly less than the changes from the basal to the placebo (paired $\underline{t} = 2.68$, $p < 0.05$). The VPB frequency rose during the placebo period compared to the (night and early morning) basal rate in 8 of the 10 patients, with a mean change of +28 VPB's per hour (for a 45 percent increase). In the hydralazine group VPB frequency fell from the basal levels, by an average of five VPB's per hour (figure 1). Because of the small number of complex ventricular arrhythmias during the study, changes in complex forms were too infrequent for meaningful analysis. There was, however, a trend toward a reduction in the number of complex VPB's during the hydralazine period as compared to the placebo period.

Although the patients in this study were selected on the basis of cardiomegaly and/or depressed left ventricular ejection fraction, resting cardiac output and pulmonary pressures in the five patients studied were normal or almost normal. Basal cardiac index averaged 2.9 $l/min/M^2$ (with a range of 2.4 to 3.1 $l/min/M^2$). Pulmonary "capillary" wedge pressure averaged 8 mm Hg (with a range of 6 to 11 mm Hg). Mean pulmonary artery pressure was 16 mm Hg (with a range of 12 to 23 mm Hg), and mean right atrial pressure was 4 mm Hg (with a range of 3 to 7 mm Hg). These measurements did not change appreciably during the administration of hydralazine or the placebo. Mean arterial blood pressure likewise did not change, averaging 95 mm Hg in the 10 patients. The average calculated systemic vascular resistance in the basal condition was 1,260 dynes-sec-cm^{-5}; this also did not vary significantly during either study period, although there was a tendency toward a reduction following administration of hydralazine. Heart rate in the basal periods averaged 69 beats/min and was usually stable following both hydralazine and the placebo.

TABLE 1. Mean Ventricular Ectopy (VPB's Per Hour) During Each Study Period

Patient	Basal*	Placebo	Change	Basal**	Hydralazine	Change
			Placebo - Basal			Hydralazine - Basal
1	19	15	-4	34	25	-9
2	51	72	+21	116	101	-15
3	102	168	+66	182	122	-60
4	21	33	+12	57	34	-23
5	4	35	+31	14	42	+28
6	24	21	-3	21	14	-7
7	26	38	+12	19	24	+5
8	111	240	+129	103	170	+67
9	275	286	+11	212	172	-40
10	2	11	+9	26	31	+5
Average ± standard deviation	63.5 ± 83	91.9 ± 102	28.4 ± 41	78.4 ± 72	73.5 ± 62	-4.9 ± 35

* Basal period on day of placebo.
** Basal period on day of hydralazine.

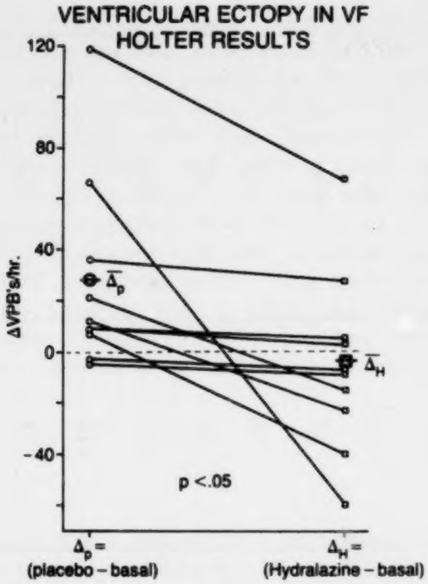

FIGURE 1. Changes in the average hourly rate of ventricular premature beats (VPB's) compared to the basal levels on 2 separate days. The reduction with hydralazine was consistently greater. The individual rates of VPB's are shown in table 1.

Although left ventricular ejection fraction did not change with the administration of hydralazine or the placebo (an average of 39 percent in the basal state), there were significant reductions in the relative ventricular volumes with hydralazine at end systole and at end diastole; these reductions averaged about 10 percent for the left ventricle and 15 percent for the right ventricle. During the placebo periods, counts at end systole and at end diastole were unchanged from basal levels. Changes from the basal condition to placebo versus changes from the basal condition to hydralazine were significant statistically using Student's paired t-test ($p < 0.05$) (figures 2 and 3).

There were no apparent side effects of hydralazine or the placebo during the study. No patient was able to determine on which day he received the active drug. One episode of angina occurred during the study. This occurred during the placebo period and required one dose of nitroglycerine.

DISCUSSION

Under the conditions of this study, there was a significant reduction of VPB's following administration of hydralazine compared to the changes after administration of a placebo. This reduction was mainly an attenuation of the expected (14) diurnal rise in ectopy which was observed on the day of placebo administration. Recent reports have reviewed the difficulties in assessing the significance of changes in ventricular ectopic activity

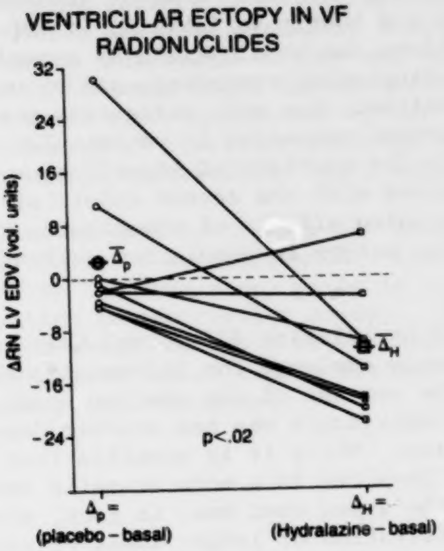

FIGURE 2. Changes in radionuclide left ventricular end-diastolic volume (RN LV EDV) corrected counts following administration of a placebo and hydralazine. The placebo and hydralazine were administered on separate days and in alternate order. There was a significantly greater reduction in counts following hydralazine compared to the changes following placebo.

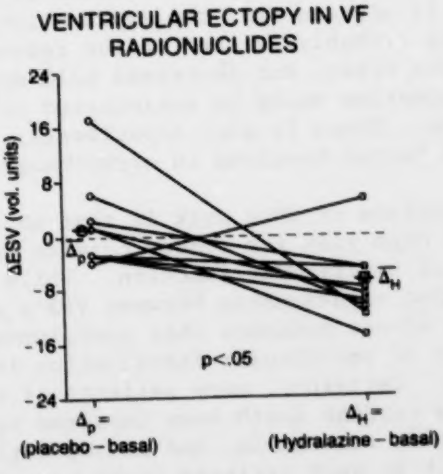

FIGURE 3. Changes in end-systolic volume (ESV) corrected counts following administration of a placebo and hydralazine. See legend for figure 2 for method of administration.

during long-term monitoring (15,16). A major problem relates to the substantial spontaneous daily and hourly variability in VPB rate. For investigative purposes these limitations can be minimized by grouping patients, by adequate duration of electrocardiographic recording, and by utilization of suitable control periods. We believe that such safeguards were accomplished in our study and that the observed reduction in ventricular ectopy satisfied reasonable requirements for statistical significance. Furthermore, our observations are in accord with the recent report of Kumpuris and colleagues (17). These authors studied effects of vasodilator therapy and observed a reduction in ventricular ectopy in ambulatory patients with congestive cardiomyopathy.

The total doses of hydralazine (35-75 mg) used in our study were small compared to those commonly employed for the treatment of congestive heart failure. The reason for our use of the smaller doses was primarily one of concern for safety in individuals who had neither demonstrable heart failure nor arterial hypertension. While it is possible that larger doses of hydralazine might have resulted in a more dramatic decrease in ventricular ectopy, the effect of the doses used was, in fact, statistically significant. Furthermore, the administration of larger doses of vasoactive drugs has the potential for producing arterial hypotension and tachycardia with a resultant deleterious effect on patients with coronary artery stenosis and near-normal resting hemodynamics.

Relationship of Ventricular Ectopy to Left Ventricular Function

The finding of a reduction in ventricular ectopy by hydralazine is provocative. In planning the study we anticipated that our patients would have substantial impairment of ventricular function at rest. Since this was not the case, a major improvement in ventricular function could not occur. Nevertheless, it appears that the explanation for the observed reduction in ectopy was probably related to the reduced heart size. The exact mechanisms are not clear, but decreased wall stress and lowered myocardial oxygen consumption would be anticipated to result from a reduction of heart size. There is also experimental evidence that myocardial fiber stretch may be a factor involved in arrhythmogenesis (8,9).

One of the implications of this work is that an antiarrhythmic regimen in certain patients at high risk for sudden cardiac death might be effected by altering the state of ventricular function. While many investigators have demonstrated a close relationship between VPB's and subsequent sudden death, there is little direct evidence that containment of VPB's will prevent the development of ventricular fibrillation in patients with chronic ischemic heart disease. Certainly, many patients at risk for ventricular fibrillation and sudden cardiac death have impaired ventricular function--some severely so. Hence, it is reasonable, and indeed logical, to consider that an antiarrhythmic effect in such patients might be mediated through the control of congestive heart failure and reduction of ventricular dilation.

As noted above, complex or high frequency VPB's in patients with ischemic heart disease appear to be harbingers of sudden cardiac death. It is unclear, however, whether VPB's represent independent markers or whether they are merely reflective of underlying structural or functional changes in the myocardium. When this question has been examined, investigators have observed a correlation between ventricular ectopy and left ventricular dysfunction in patients with ischemic heart disease (7,18). The observations reported in this study indicate that ventricular ectopy can be attenuated by altering ventricular function, thus providing evidence for a dependent relationship between ventricular function and VPB's under some circumstances.

We believe that further studies to clarify the antiarrhythmic effect of improving left ventricular function are warranted. Controlled short-term trials of vasodilator therapy for ambulatory patients would be helpful to validate a sustained reduction in ventricular ectopy. If these findings are borne out, consideration should be given to implementation of long-term trials directed toward the prevention of sudden cardiac death by use of vasodilating agents in appropriate patients.

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IDENTIFICATION OF PATIENTS AT HIGH RISK OF SUDDEN DEATH BY EXERCISE TESTING AND 24-HOUR ECG MONITORING

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ABSTRACT. The prognostic significance of 24-hour electrocardiographic (ECG) monitoring and bicycle exercise testing was studied in 300 patients with established ischemic heart disease (IHD). Twenty-one cases of sudden death (7 percent) occurred during the 2-year followup period. The signs most strongly associated with subsequent sudden death were: repetitive (couplets and runs) ventricular extrasystoles (VE) during monitoring, persistence of ventricular arrhythmias (recorded for 8 hours or more in a 24-hour monitoring period), frequent and complex VE during the exercise test, reduced maximal heart rate during exercise, and a positive exercise test. Groups of patients at a very high and very low risk of sudden death were identified using conjunction and inclusive dysjunction of the above indexes. The patients with the poorest prognosis were those with "threatening" arrhythmias found during the 24-hour monitoring or during the exercise test, and who also had a positive test and reduced aerobic capacity. Sixty percent of these patients died suddenly within the 2 years of followup. Among patients with a negative exercise test, only those with VE during both prolonged monitoring and bicycle exercise died suddenly during the 2 years; in most of these cases, the determining factors were frequent or complex VE recorded throughout most of the monitoring session. Some of the patients who died suddenly had neither "threatening" nor persistent ventricular arrhythmias, but in all of these cases a high degree of abnormal systolic blood pressure response to exercise or ST-segment depression had been noted. Successive (multistage) risk stratification resulted in identification of a group consisting of only one-third of the patients studied, but including all of the subsequent cases of sudden death.

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INTRODUCTION

It has been recently shown that the extent and severity of coronary artery disease and the degree of disorders in the contractile capacity of the myocardium are important risk factors for sudden cardiac death in patients with IHD. Another risk factor of definite prognostic significance as a precursor of fibrillation is ventricular extrasystole. To reveal ventricular arrhythmias, prolonged ECG monitoring and exercise stress testing have been used lately; the latter method is also being used to assess indirectly the condition of the coronary arteries and the contractile capacity of the myocardium. We studied the feasibility of using these two methods for identifying persons at high risk of sudden death among patients with established IHD.

MATERIALS AND METHODS

The study involved 300 men 31 to 64 years of age who had suffered a myocardial infarction at least 3 months before the study began; the mean age of the subjects was 50 years. An ECG was recorded in one lead using portable monitors (American Optical Co.) for 24 hours as the subjects continued their normal daily activity. Recordings were subsequently interpreted on an analyzer (made by the same firm) by two physicians independently using audiovisual testing. Furthermore, all patients were given a bicycle exercise test. During the bicycle test and subsequent rest of 10 minutes, the ECG was recorded continuously on magnetic tape. The ECG was also recorded on paper tape for 10 seconds of each minute of the study, including the 10-minute recovery period. Blood pressure was measured before exercise, during the first and the last minute at each stage of exercise, and several times during the recovery period.

The exercise test was conducted by staged increases in load, beginning at 150 kgm/min and increasing every 5 minutes by 150 kgm/min. The load was increased until the patient reached a heart rate of 75 percent of the maximum aerobic capacity, i.e., approximately 85 percent of the maximum heart rate predicted on the basis of the patient's age. The test was interrupted if angina, pain in the gastrocnemius muscles, or fatigue appeared; if the blood pressure rose above 220/120 mm Hg; or if there was a pathologic blood pressure response, i.e., a drop in systolic pressure by 10 mm Hg or more in comparison to the previous measurement, no increase in systolic pressure (identical pressure at two stages of load), or if 1 mm or greater horizontal or down-sloping ST-segment depression developed for at least 0.08 second and frequent, repetitive (couplets or runs) or early type R/T extrasystoles were recorded. The ECG monitoring was performed before the bicycle test, and the exercise test was conducted no more than 5 days after a 24-hour ECG recording.

All 300 patients were followed up for 2 years, during which time all cardiac outcome was recorded. The present report only analyzes cases of sudden coronary death, defined as "unexpected" death within 6 hours from the onset of symptoms.

In order to identify groups of persons at high risk of sudden death, we evaluated the significance of the following indexes during the study:

- Presence of VE during ECG monitoring or exercise, involving complex rhythm disorders (multifocal extrasystoles, repetitive, and early type R/T extrasystoles) and frequent VE. Frequent extrasystoles were defined during bicycle ergometry as three or more extrasystoles per minute, and during constant ECG monitoring as more than 30 extrasystoles per hour.
- Number of hours during continuous monitoring in which VE were recorded.
- Appearance of ST-segment depression or abnormal blood pressure response during exercise (i.e., a positive exercise test).
- Maximum systolic pressure and maximum heart rate during exercise.

All of these indexes were analyzed by prognostic stratification, a method which has been used previously and described by Bigger et al. (1). Prognostic stratification was applied both to the univariates and to their combinations. We grouped the indexes into combinations using conjunction ("&") and (inclusive) dysjunction ("V").

Conjunction-type combinations are those in which a patient had all of the indexes in the combination. Dysjunction-type combinations are those in which the patient had at least one of the indexes in the combination. Some combinations included both the conjunction and dysjunction of indexes.

Prognostic stratification applied to the variables of prolonged ECG monitoring and the exercise test resulted in the construction of a 2 x 2 matrix for each index and for each combination of indexes. Sensitivity, specificity, predictive accuracy, and relative risk were calculated for each 2 x 2 matrix (see table 1). To determine the significance of association between characteristics and outcome, chi squares were calculated.

TABLE 1. Matrix (2 x 2) for Stratification of Patients in Terms of Risk of Sudden Death

Index, or combination of indexes	Sudden death	
	Present	Absent
Present	True positive	False positive
Absent	False negative	True negative

Sensitivity equaled $\frac{\text{true positive}}{\text{true positive + false negative}}$ x 100.

$$\text{Specificity equaled } \frac{\text{true negative}}{\text{true negative + false positive}} \times 100.$$

$$\text{Predictive accuracy equaled } \frac{\text{true positive}}{\text{true positive + false positive}} \times 100.$$

$$\text{Relative risk equaled } \frac{\text{true positive/true positive + false positive}}{\text{false negative/false negative + true negative.}}$$

RESULTS

During the 2 year followup, 21 cases of sudden death (7.0 percent) were reported in the study group of 300 patients. In analyzing the prognostic significance of VE found during prolonged ECG monitoring and exercise testing, we observed that the simple presence of VE was not significantly associated with sudden death over the 2 years (chi-squares were equal to 3.42 and 2.54, respectively). This is explained by the very low specificity of the prognosis based on ECG monitoring and the insufficiently high sensitivity of the prognosis based on bicycle ergometry. Thus, in spite of the fact that all of the victims had ventricular arrhythmias in their 24-hour ECG's, these were also recorded in the overwhelming majority of all test subjects (83 percent). On the other hand, although the lethality in patients with VE induced by exercise was somewhat higher than in patients with VE in the 24-hour ECG (9.8 percent and 8.4 percent, respectively), nine (or 43 percent) of the sudden death victims had no VE during the exercise test.

All of the univariates that were significantly associated with sudden death within 2 years are presented in table 2. The table shows that the highest sudden death rates were observed in patients with repetitive VE during prolonged ECG monitoring and in those with exercise-induced frequent and complex arrhythmias (predictive accuracy of 19.4 percent and 16.7 percent, respectively). We have defined such arrhythmias as "threatening." However, multifactorial analysis has demonstrated that neither of these indexes can be considered independent risk factors for sudden death. The prognosis for patients with threatening arrhythmias depends to a great extent on whether they also have other indexes recorded during the study. For example, as can be seen from figure 1, patients with repetitive extrasystoles in the 24-hour ECG monitoring can be divided into two groups: (a) those in whom the exercise test was stopped before the heart rate rose above 115/minute; and (b) those who reached a higher heart rate during exercise testing. In the first group we recorded 10 cases of sudden death out of 28 subjects (35.7 percent), whereas the lethality in the second group was only 5.9 percent (two cases of sudden death among 34 subjects)--only slightly greater than the lethality in the patients without repetitive VE during prolonged monitoring (nine cases of sudden death among 238 subjects, or 3.8 percent).

An analogous picture was also observed in patients with frequent and complex arrhythmias induced by exercise. Figure 2 shows that of 23 patients with these rhythm disorders, in whom the test was stopped before a heart rate above 115/minute was reached, 8 died suddenly (34.8 percent), whereas in a group of 31 patients with frequent and complex arrhythmias during bicycle ergometry,

TABLE 2. Prognostic Significance of Exercise and Monitoring Univariates for Sudden Death Within 2 Years of Followup

No.	Univariate	2 x 2 Matrix	Predictive accuracy (%)	Relative risk	Sensitivity (%)	Specificity (%)	X^2
1	PM_{repVE}	$\frac{12}{9}\ \frac{50}{229}$	19.4	5	57	82	18.32 ($p < 0.001$)
2	$ET_{MHR \le 115'}$	$\frac{16}{5}\ \frac{88}{191}$	15.4	6	76	68	15.28 ($p < 0.001$)
3	ET_{serVE*}	$\frac{9}{12}\ \frac{45}{234}$	16.7	3.5	43	84	9.45 ($p < 0.01$)
4	$PM_{VE \ge 8\ hr}$	$\frac{15}{6}\ \frac{109}{170}$	12.1	3.5	71	61	8.43 ($p < 0.01$)
5	ET_{pos}	$\frac{17}{4}\ \frac{152}{127}$	10.1	3.5	81	46	4.54 ($p < 0.05$)
6	PM_{mulVE}	$\frac{15}{6}\ \frac{135}{144}$	10.0	2.5	71	52	4.15 ($p < 0.05$)
7	$PM_{VE \ge 15\ hr}$	$\frac{9}{12}\ \frac{65}{214}$	12.2	2.5	43	77	4.02 ($p < 0.05$)

PM_{repVE} = Repetitive (two or more sequential) VE during prolonged ECG monitoring.

$ET_{MHR \le 115'}$ = Maximum heart rate during exercise not over 115 beats/minute.

ET_{serVE} = Frequent (≥ 3/min) and complex VE (multifocal, repetitive, and early type R/T) during exercise.

$PM_{VE \ge 8\ hr}$ = VE in at least 8 hours of the 24-hour monitoring.

ET_{pos} = Positive exercise test.

PM_{mulVE} = Multifocal VE during ECG monitoring.

$PM_{VE \ge 15\ hr}$ = VE in at least 15 hours of the 24-hour monitoring.

*During bicycle ergometry frequent, multifocal, repetitive, and early extrasystoles were found rather rarely, and therefore these types of rhythm disorders were not analyzed individually.

but with greater aerobic capacity, only 1 case of sudden death (3.2 percent) occurred; in this latter group the lethality was even lower than in the group of patients without threatening arrhythmias induced by exercise (4.9 percent).

As our analysis showed, the prognosis for patients with threatening arrhythmias also depends to a great extent on whether the patients manifest with exercise ST-segment depression or an abnormal reaction of systolic pressure. Thus, analysis of various pairs of indexes enabled us to identify groups of patients at higher risk of sudden death than did analysis of univariates.

Risk stratification was applied to all possible bivariate and trivariate combinations of monitoring and exercise variables. Use of combinations of the conjunction type allowed us to distinguish groups of patients at a very high

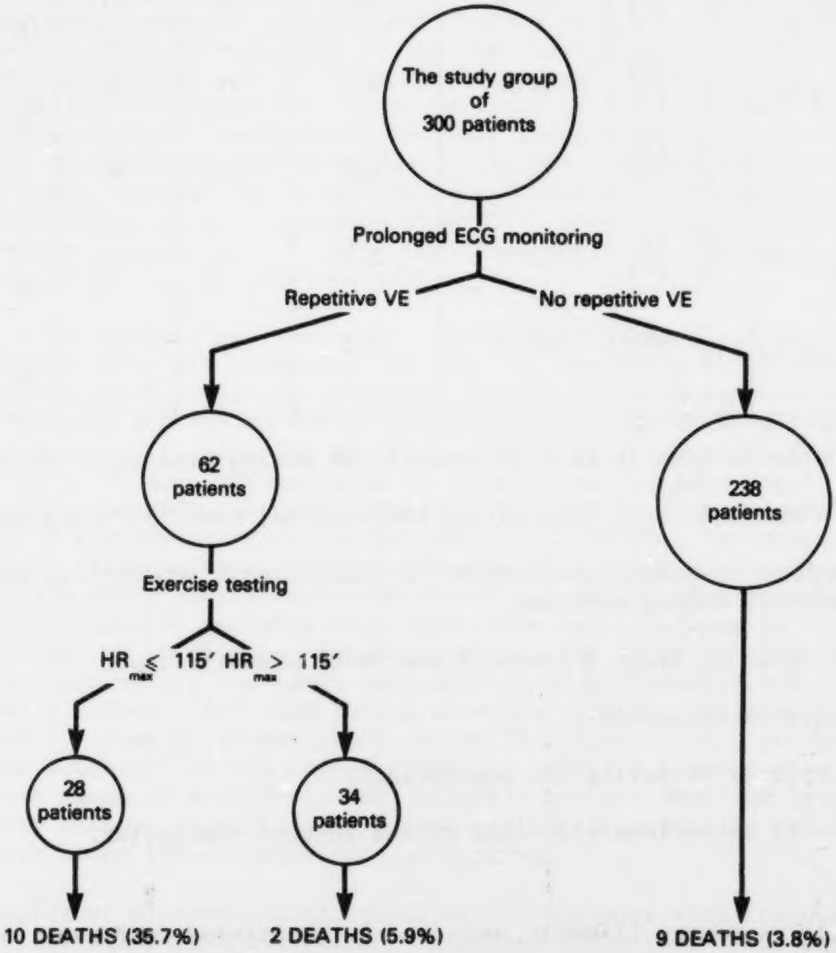
The study group of 300 patients
Prolonged ECG monitoring
Repetitive VE
No repetitive VE
62 patients
238 patients
Exercise testing
HR max ≤ 115′
HR max > 115′
28 patients
34 patients
10 DEATHS (35.7%)
2 DEATHS (5.9%)
9 DEATHS (3.8%)

FIGURE 1. Sudden death rate in patients with repetitive ventricular extrasystoles revealed by prolonged monitoring, depending on their aerobic capacity.

risk of sudden death. As the present study showed, the poorest prognosis was for the patients with a positive exercise test, decreased aerobic capacity, and (a) repetitive extrasystoles during the 24-hour ECG monitoring, or (b) frequent and complex VE during the exercise test. The prognostic significance of those trivariates is illustrated by table 3.

The table shows that both trivariates are characterized by a very high predictive accuracy (60 and 70 percent, respectively). However, in neither instance did we identify all of the patients who died suddenly. More than half of the sudden death victims did not have a positive exercise test with reduced aerobic capacity in combination with repetitive extrasystoles on the 24-hour ECG, and two-thirds of the sudden death victims did not have a positive exercise test with simultaneous frequent and complex VE and reduced aerobic capacity. This is not surprising. On the one hand, it is natural that the

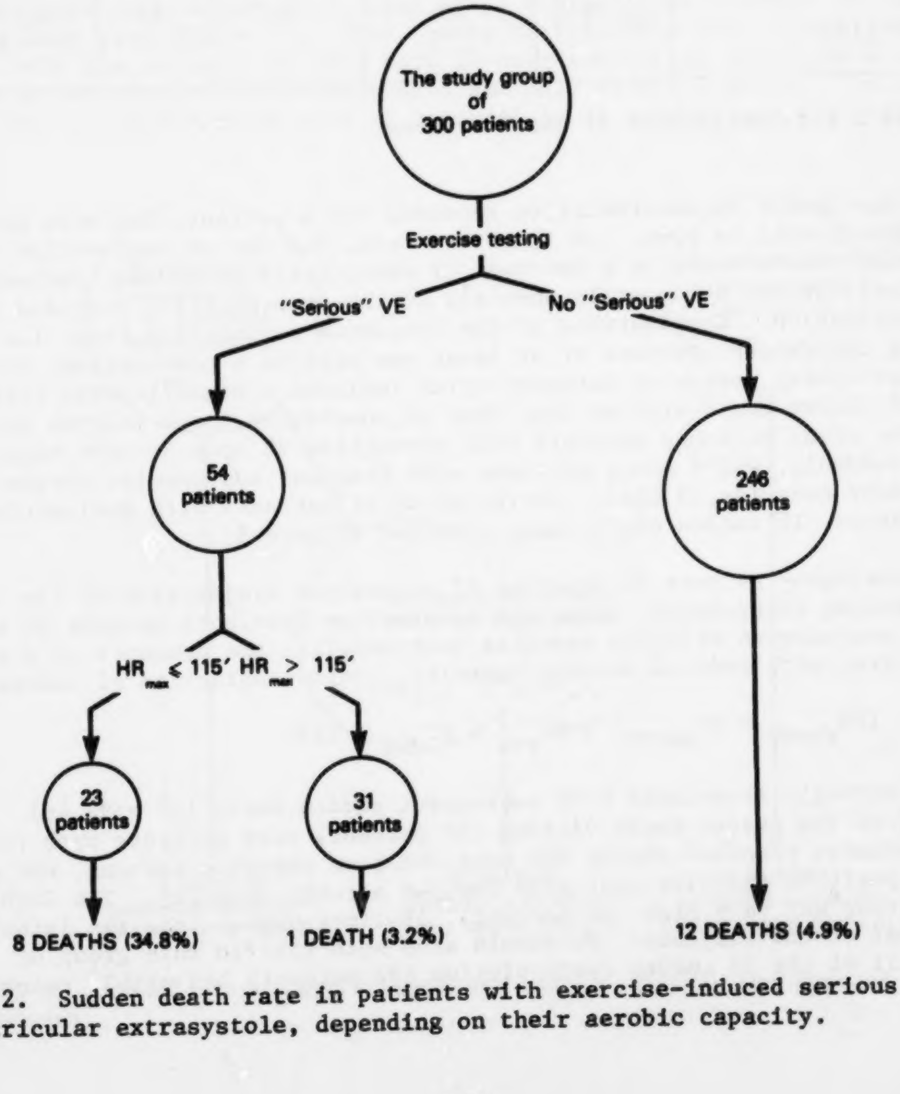
The study group of 300 patients
Exercise testing
"Serious" VE
No "Serious" VE
54 patients
246 patients
HR$_{max}$ ≤ 115′
HR$_{max}$ > 115′
23 patients
31 patients
8 DEATHS (34.8%)
1 DEATH (3.2%)
12 DEATHS (4.9%)

FIGURE 2. Sudden death rate in patients with exercise-induced serious forms of ventricular extrasystole, depending on their aerobic capacity.

TABLE 3. Prognostic Significance of Trivariates for Identifying Groups at a Very High Risk of Sudden Death

Combination of indexes	2 x 2 matrix	Predictive accuracy (%)	Relative risk	Sensitivity (%)	Specificity (%)	χ^2
PM_{repVE} and ET_{pos} and $ET_{MHR} \le 115'$	$\frac{9}{12}\ \frac{6}{273}$	60	14	43	98	68.13
ET_{serVE} and ET_{pos} and $ET_{MHR} \le 115'$	$\frac{7}{14}\ \frac{3}{276}$	70	15	33	99	53.46

See table 2 for designation of abbreviations.

greater the number of abnormalities recorded for a patient, the more probable his prognosis will be poor. On the other hand, the use of conjunction type combinations alone leads to a decrease of sensitivity since many patients who die suddenly do not necessarily have all of the abnormalities included in a given combination. Combinations of the inclusive dysjunction type, i.e., requiring obligatory presence of at least one sign in a combination, allowed us to distinguish groups of patients which included a significantly larger number of sudden death victims than when we considered these indexes individually. For example, among patients with repetitive VE upon 24-hour monitoring, 12 died suddenly, while among patients with frequent and complex rhythm disorders under exercise, 9 died. In the group of patients with dysjunction of these indexes, 16 sudden death cases occurred (figure 3).

An analogous picture is observed if we analyze dysjunction of the indexes of threatening arrhythmias, using the information from both methods of examination, in conjunction with the exercise test results: the presence of a positive exercise test with reduced aerobic capacity. This combination of indexes,

$$(PM_{repVE} \vee ET_{serVE}) \mathbin{\&} ET_{pos} \mathbin{\&} ET_{MHR} \le 115'$$

was most strongly associated with subsequent sudden death ($\chi^2 = 92.46$). More than half of the sudden death victims (57 percent) were patients with threatening arrhythmias recorded during ECG monitoring or exercise testing, who also showed a positive exercise test with reduced aerobic capacity. The lethality of this group was very high (60 percent), 19 times higher than the lethality of the rest of the subjects. We should also note that in this group of patients 11 of the 12 sudden death victims (92 percent) had stable recordings of ventricular arrhythmia (in the ECG monitoring, VE were recorded in 8 or more hours). The data are presented in figure 4.

Of the 280 subjects who did not have this combination of indexes, prognosis was comparatively favorable (and lethality averaged 3.2 percent). Further analysis revealed combinations of characteristics which identified patients at elevated risk of sudden death in this group as well.

We divided the patients into those with no ST-segment depression or abnormal response of blood pressure with exercise, and those with a positive exercise test.

Among these patients, 131 had no positive exercise test; four cases of sudden death (3.1 percent) subsequently occurred in this group. All four cases were in a subgroup of 39 subjects with VE both during ECG monitoring and during the exercise test (lethality of 10.3 percent), whereas none of the 92 patients without this combination of indexes (PM_{VE} & ET_{VE}) died suddenly in the 2 years of followup (see figure 4). Thus, among the patients with a negative exercise test, only the patients in whom both 24-hour monitoring and bicycle ergometry revealed ventricular arrhythmias died suddenly within 2 years. The poorest prognosis was for persons with VE recorded in 15 or more hours of ECG monitoring,

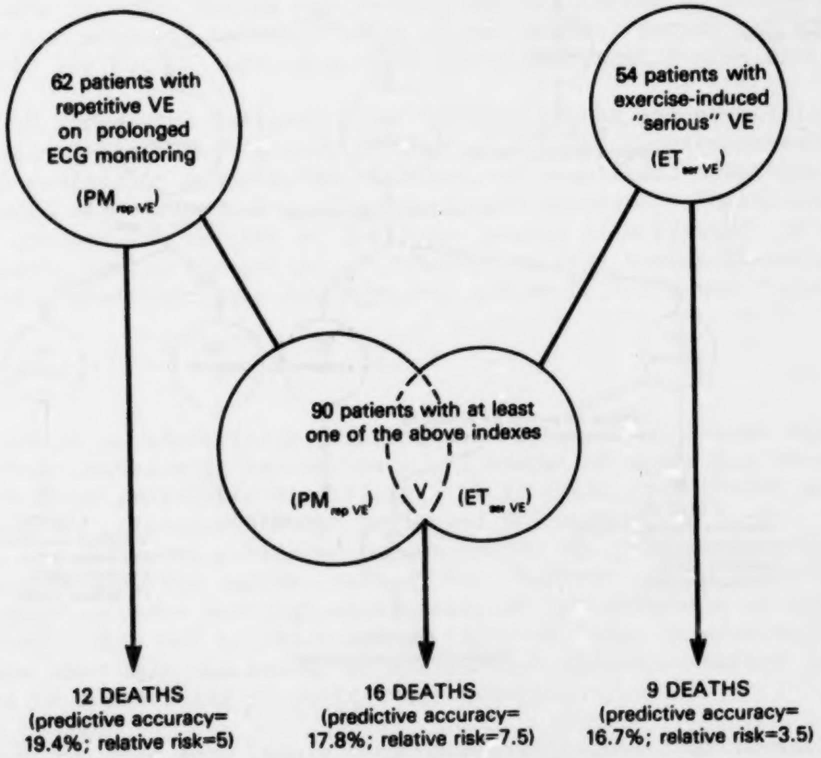
62 patients with repetitive VE on prolonged ECG monitoring
($PM_{rep\ VE}$)
54 patients with exercise-induced "serious" VE
($ET_{ser\ VE}$)
90 patients with at least one of the above indexes
($PM_{rep\ VE}$) V ($ET_{ser\ VE}$)
12 DEATHS (predictive accuracy= 19.4%; relative risk=5)
16 DEATHS (predictive accuracy= 17.8%; relative risk=7.5)
9 DEATHS (predictive accuracy= 16.7%; relative risk=3.5)

FIGURE 3. Sudden death rate in patients with a dysjunction type of combination of indexes.

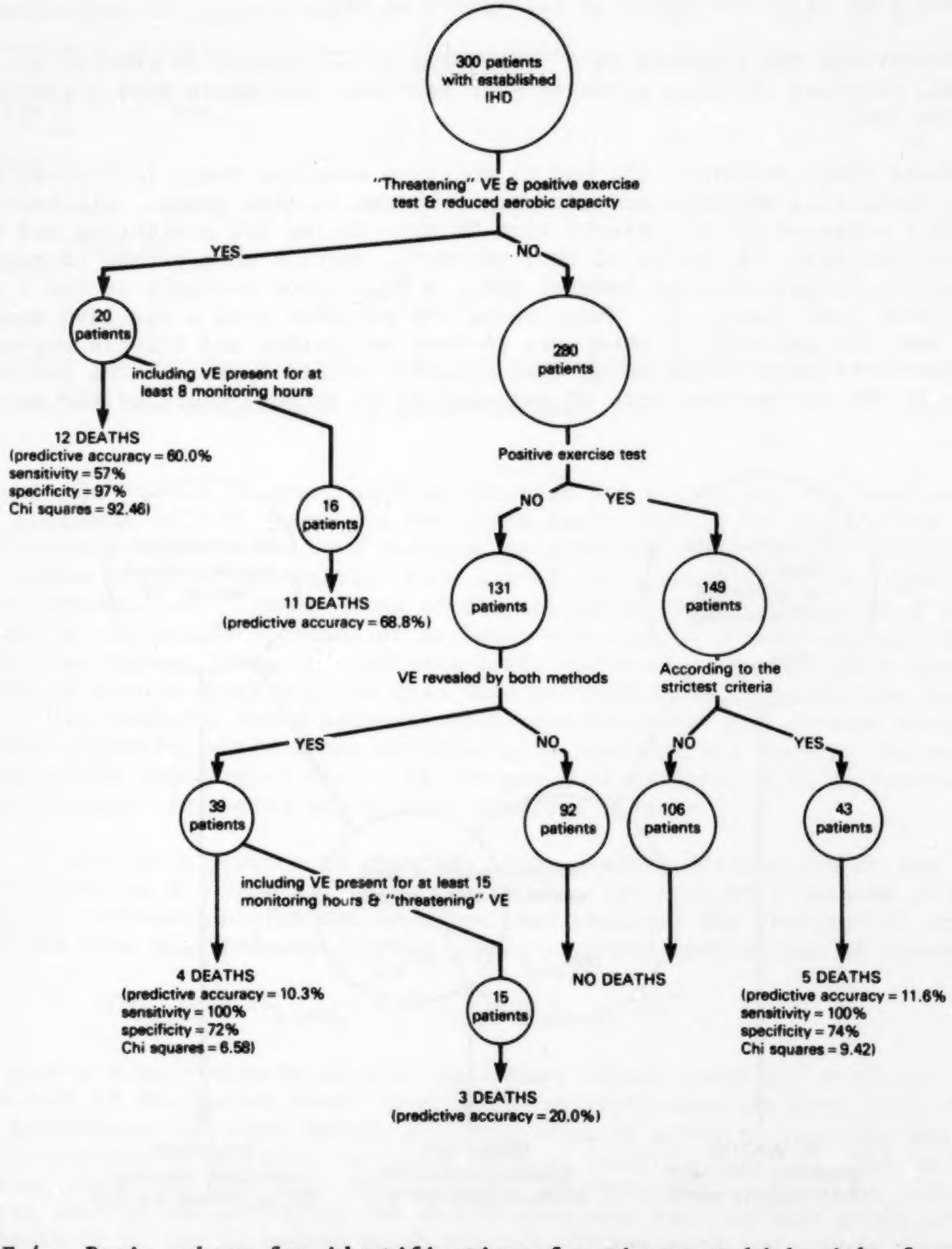
300 patients with established IHD
"Threatening" VE & positive exercise test & reduced aerobic capacity
YES
NO
20 patients
including VE present for at least 8 monitoring hours
12 DEATHS
(predictive accuracy = 60.0%
sensitivity = 57%
specificity = 97%
Chi squares = 92.46)
16 patients
11 DEATHS
(predictive accuracy = 68.8%)
280 patients
Positive exercise test
NO
YES
131 patients
149 patients
VE revealed by both methods
According to the strictest criteria
YES
NO
NO
YES
39 patients
92 patients
106 patients
43 patients
including VE present for at least 15 monitoring hours & "threatening" VE
4 DEATHS
(predictive accuracy = 10.3%
sensitivity = 100%
specificity = 72%
Chi squares = 6.58)
15 patients
3 DEATHS
(predictive accuracy = 20.0%)
NO DEATHS
5 DEATHS
(predictive accuracy = 11.6%
sensitivity = 100%
specificity = 74%
Chi squares = 9.42)

FIGURE 4. Basic scheme for identification of patients at high risk of sudden death.

and with threatening arrhythmias revealed by one of the two test methods (see figure 4).

Among the 149 patients with positive exercise tests, five cases of sudden death (3.4 percent) occurred in the 2 years of subsequent followup. More rigid criteria for a positive test were required to identify these persons:

- ST-segment depression only with reduced aerobic capacity (maximum heart rate with exercise not greater than 115/min)
- Significant drop in systolic pressure with exercise (by at least 25 mm Hg)
- No rise in systolic pressure with relatively low maximum pressure with exercise (≤ 165 mm Hg).

The dysjunction of these characteristics revealed a group of patients with a relatively high lethality (11.6 percent), whereas no cases of sudden death occurred in patients without these characteristics (see figure 4).

It should be noted that rhythm disorders were seen relatively infrequently in this group of patients. Thus, in three of the five patients who died suddenly, VE were recorded during the 24-hour ECG monitoring in only 1 or 2 hours; only one of the subjects had repetitive VE (a couplet) during the ECG monitoring, and only one patient experienced VE (rare, monotopic) with exercise.

Thus, by successive delineation of patient groups and stratification of risk of sudden death based on combinations of various indexes revealed in the 24-hour ECG monitoring and exercise testing, we identified subgroups of patients which included all of the cases of sudden death observed. These subgroups included 102 persons (34 percent of the total number of subjects), of whom 1 in 5 died suddenly during the period of observation (the predictive accuracy was 20.6 percent, sensitivity was 100 percent, and specificity was 71 percent).

DISCUSSION

The problem of identifying persons at a high risk of sudden death has received great emphasis in recent years. A number of works has been published which discuss the prognostic significance of VE found in patients with IHD by various methods, primarily through prolonged ECG monitoring (2-5). A number of studies has also been published on evaluating the prognostic significance of the exercise test for sudden death (6-9). However, in no study known to us have both high accuracy and high sensitivity of the prognosis of sudden death been achieved. This may be explained by the fact that, in most works, the authors have used only one method of examination and have limited themselves to analysis of univariates or their conjunctions.

It is known that some highly prognostic indicators (particularly threatening arrhythmias) are not necessarily exposed in a patient by both prolonged ECG monitoring and exercise testing (9,10). One may assume, therefore, that these are complementary methods for identifying patients at high risk of sudden death.

The results of our study, which included both examination methods, have confirmed this assumption. Using various combinations of indexes from both methods, in both conjunction and dysjunction, we succeeded in identifying a group of patients at a very high risk of sudden death. The prognosis of sudden death is not only highly accurate, but also highly sensitive. According to our study, the patients with the poorest prognosis had threatening arrhythmias, ST-segment depression or an abnormal response of systolic pressure during exercise, and reduced aerobic capacity. Sixty percent of the patients with this combination of indexes, as determined by the two examination methods, died from sudden death within 2 years, and more than half of all sudden death victims manifested this pathology (sensitivity was 57 percent).

Many studies have indicated that the greater the number of pathological symptoms found with exercise testing, the greater the probability that there is significant, extensive damage of the coronary arteries (8,11,12). Based on these data, we can presume that patients with this combination of indexes determined by ECG monitoring and exercise testing also have clearly ectopic myocardial activity and significant damage to the coronary arteries. It is in these patients that we found the highest lethality.

The overwhelming majority of the subjects (280 patients) did not have such clearly manifest pathology, but even among these, 9 died suddenly. Our methods of examination and data analysis allowed us to find combinations of indexes which identified persons at a high risk of sudden death even among these 280 patients.

It has been shown that significant coronary stenosis, although frequently present, is unnecessary for the development of fatal arrhythmias (13-15). Our study has shown that among patients with negative exercise tests, only those with VE exposed by both methods of examination die suddenly; in most cases, these subjects manifested frequent, repetitive, and stable extrasystoles, recorded in at least 15 hours of monitoring. We can assume, therefore, that in patients without severe coronary artery damage (indirectly indicated by the absence of an ischemic ST-segment depression and abnormal blood pressure response with exercise), a well-defined (that is, clearly expressed) myocardial electrical instability is necessary for the development of fatal arrhythmias.

Sudden death victims also included patients without threatening or persistent ventricular arrhythmias, but all of these subjects had clearly expressed deviations in several characteristics during the exercise test. ST-segment depression appeared in these patients with a low heart rate, a drop in systolic pressure from the highest level attained was of at least 25 mm Hg, and a flat response in systolic pressure was observed at its relatively low level (not over 165 mm Hg). The first two abnormalities have long been considered the indicators of severe coronary artery disease (11,16), while the latter characteristic has been also associated with severe irreversible myocardial dysfunction (17). It can be assumed, therefore, that in patients with a significant coronary stenosis and impaired left ventricular function the persistence of ventricular ectopy is unnecessary for the development of fatal arrhythmias. Longer monitoring sessions or repeated examinations should be recommended for such patients to reveal threatening arrhythmias as harbingers of ventricular fibrillation.

CONCLUSIONS

1. Using various exercise testing and prolonged ECG monitoring indexes, persons at a very high and very low risk of sudden death can be identified among patients with IHD with a history of myocardial infarction. The two methods complement each other, and the greater the number of abnormalities revealed by these examination methods, the greater the probability that a patient is a potential sudden death victim.

2. The highest risk of sudden death was found among patients with threatening arrhythmias recorded during the exercise test or 24-hour ECG monitoring, and with positive exercise tests and reduced aerobic capacity. The prognosis of sudden death based on this combination of indexes is not only highly accurate (60 percent), but also very sensitive (57 percent).

3. Almost 20 percent of the sudden death victims did not have a positive exercise test; however, in all of these patients ventricular arrhythmia was seen both during bicycle ergometry and during ECG monitoring, and most of these patients experienced threatening VE in 15 or more of the 24 hours of monitoring.

4. The sudden death victims also included patients without threatening or persistent arrhythmias. However, in all of these patients the exercise test was positive in accordance with the strictest criteria.

5. Stratification of patients in terms of their risk of sudden death allowed us to identify three subgroups which accounted for only one-third of all subjects, but included all who later died suddenly.

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REFINEMENTS OF AN AUTOMATED HOLTER TAPE REVIEW SYSTEM

Suzanne B. Knoebel and D. Eugene Lovelace

ABSTRACT. Automated ambulatory electrocardiographic (ECG) review systems must provide for the identification of multiform ventricular complexes and must provide for noise rejection. The Krannert Institute Computer System, which classifies ventricular ectopic complexes (VEC) on the basis of QRS amplitude, R-R interval, and ST segment slope, has recently been modified to include (a) identification of multiform VEC on the basis of two-dimensional clustering of R-R interval and early T-wave slope, and (b) noise rejection through recognition of deviation from "normal" ST amplitude sequencing. Preliminary results from 25 patients having high frequency of occurrence of VEC and high noise levels indicate that the algorithm correctly identified the presence of multiform VEC in all patients and that there were no false positive multiform identifications. Application of the noise rejection criteria reduced false positive VEC identification to 2.3 percent in these high noise level tapes.

INTRODUCTION

The Holter monitoring technique, which permits ECG recordings over extended periods of time in ambulatory patients, was introduced in 1961 (1). While conventional methods of review of such tape recordings are adequate for clinical detection of transient major rhythm disturbances, many believe that quantitative analysis requires automated systems. In addition, in order to assess any predictive events for premature ventricular complex (PVC) emergence, large masses of data must be analyzed. Within the past

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few years, several centers have developed computer systems designed for quantitative analysis of Holter tapes. The general concepts and formats for these systems have been described by Oliver (2), who also reviewed in the same paper the rationale for and the development of systems for PVC identification.

As developments have proceeded in computer-assisted ambulatory tape analyses, it has become apparent that a prime issue for all systems is noise interference resulting in excessive false positive PVC identification. Most systems perform adequately with "clean" recordings. With increased experience with computer analysis, in conjunction with clinical studies attempting to define the prognostic significance of PVC based on morphology, it has also become apparent that provision must be made for the identification of multiform PVC.

The purpose of this paper is to describe the Krannert Institute System for computer analysis of ambulatory ECG recordings, including the algorithms for noise rejection and multiform PVC recognition (3-6). Modifications have been made in this system since previous publications.

METHODS

The Krannert Institute System for QRS identification and classification utilizes a PDP 11/34 Central Processor with 32,000 words of central memory, a 10 megabyte disk drive, a Tektronix 4010-1 graphics terminal, and a data acquisition and control subsystem (KDAC1) designed at the Krannert Institute. The data acquisition and control subsystem interfaces directly to the UNIBUS and appears as a memory module to the RSX11M operating system (figure 1). The KDAC1 module performs the basic functions of (a) A/D conversion of two channels of ECG at rates of up to 24,000 samples/sec, (b) definition of the location of each QRS complex using two channels of analog tape data, and (c) determination and storage of the values of the parameters utilized for QRS identification and classification.

QRS classification decisions are based upon the R-R interval, the weighted QRS amplitude (YDEL), and the slope of the early ST and T-wave (TDIFF) (figure 2). The intervals A and B together with the isoelectric interval YISO are fixed with respect to the fiducial point YNLOC. The isoelectric interval is from 270 msec to 200 msec before YNLOC. The interval A is from 90 msec to 130 msec after the reference point. The interval B begins 180 msec after the reference point and lasts until 230 msec. The slope of the early T-wave, denoted TDIFF, is defined using a least squares straight line fitted to the sampled ECG over the intervals A and B. The QRS amplitude is defined as a weighted average of the sampled ECG one point before to one point after the fiducial point. The QRS amplitude and TDIFF are entered into frequency of occurrence arrays which are maintained for a period of 100 complexes. The limiting values for QRS amplitude and early T-wave slope are defined from these probability density functions. The upper and lower limits must include 98 percent of the data and are updated each 100 complexes. The R-R interval duration (T_{rr}) is maintained in a similar table.

The decision table used in classifying complexes is based on the limit values for the normal sinus complex. If the complex has a normal QRS amplitude

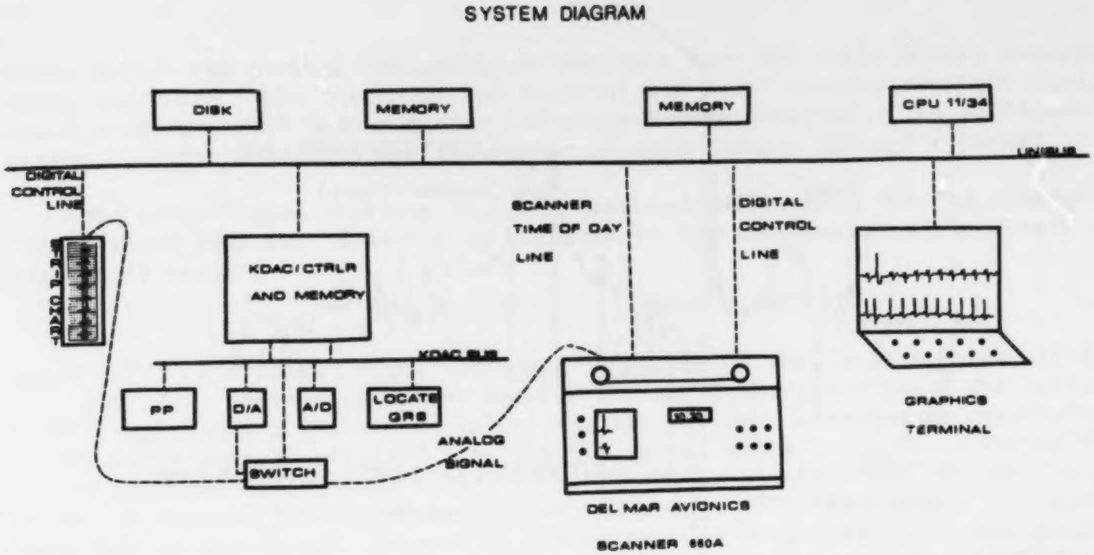
SYSTEM DIAGRAM
DISK
MEMORY
MEMORY
CPU 11/34
UNIBUS
DIGITAL CONTROL LINE
STRIP CHART
KDAC/CTRLR AND MEMORY
SCANNER TIME OF DAY LINE
DIGITAL CONTROL LINE
KDAC BUS
PP
D/A
A/D
LOCATE QRS
SWITCH
ANALOG SIGNAL
10:30
DEL MAR AVIONICS SCANNER 660A
GRAPHICS TERMINAL

FIGURE 1. System diagram. The 660A Scanner is interfaced to both the KDAC1 data acquisition system and the UNIBUS. The scanner transport controls and the time of day clock are monitored by the computer. These data are used to define the sampling rate for KDAC1 and to define the time of day. The strip chart recorder is controlled by the computer and is equipped with a printer which marks each strip with patient name and Holter number, the reason the strip was generated, and annotates the strip with computer interpretation. PP = Parameter processing. CPU = Central processor unit. CTRLR = Digital controller. A/D = Analog to digital converter. D/A = Digital to analog converter.

and a normal ST-T segment slope, it is classified as either a normal sinus or atrial premature complex depending upon the degree of prematurity. If the complex has an abnormal ST-T segment slope and an abnormal QRS amplitude parameter, the complex is termed a PVC or a VEC depending upon the degree of prematurity. The noise rejection criteria, described below, may alter this basic decision scheme.

Noise Rejection

Of particular importance to this algorithm is the TDIFF parameter. As defined above, TDIFF is the slope of a least squares straight line passed through the sampled ECG, from 90 msec after the QRS reference point to 230 msec after the QRS reference point.

The average amplitude sequence from 90 to 230 msec after the reference point (15 samples) is maintained in a vector ST(i) and updated each 25 normal sinus complexes. The noise amplitude distortion (N/S) is defined from the difference,

$$d(i) = y(i) - ST(i)$$

where y(i) = the sampled ECG value, over the same time interval as ST(i), and

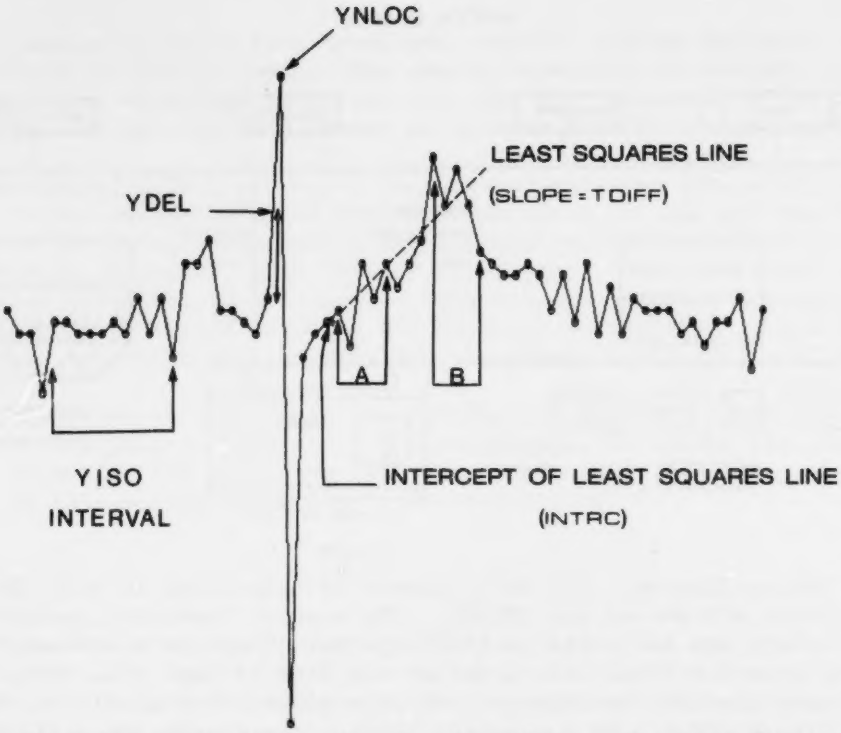

FIGURE 2. Decision parameters. 0 = Sample points. See text for explanation of symbols.

i = the index in the average amplitude vector ST(i). The difference vector d(i) is ordered from maximum to minimum and the average amplitude distortion is defined as

$$N/S = \frac{1}{P} \sum_{J=1}^{P} \hat{d}(J) - \hat{d}(16-J)$$

where $\hat{d}(J)$ and $\hat{d}(16-J)$ = rank ordered difference values for d(J), and P = number of pairs of points over which the average is taken. The range of values of the noise amplitude distortion parameter N/S is collected for a period of 100 complexes. When the QRS classification values are determined, the maximum value for N/S is also determined. This maximum value represents the limit values for noise amplitude distortion during the next 100 normal sinus complexes.

The second characteristic of noise artifact tested by this algorithm is the sudden increase in the frequency of amplitude fluctuation of the sampled ECG during the early ST-T time interval. The residual between the T-wave amplitude sequence and the least squares straight line used to define TDIFF is maintained in a vector

$$DIFF(i) = y(i) - (INTRC + TDIFF * i)$$

where y(i) = the sampled ECG amplitude sequence over the early T-wave interval, INTRC and TDIFF = the intercept and slope of the least squares straight line, and i = the interval number during the early T-wave interval. The difference vector DIFF(i) is scanned and the number of sign changes (ST_{SEG}) recorded.

The early T-wave template contains temporal information for the average normal sinus complex. A vector of differences for the average early T-wave segment is derived

$$DST(i) = ST(i) - (INTRC' + TDIFF' * i)$$

where INTRC' and TDIFF' = the intercept and slope of the least squares straight line for ST(i) defined as in the sampled ECG TDIFF determination. The vector DST(i) represents the expected values for the average early T-wave departures from a least squares straight line for the normal sinus complex. A second vector of differences, D2ST(i) = DIFF(i) - DST(i), is obtained and the resulting vector is scanned for the number of zero crossings (STDIFF). Since the expected amplitude of the normal sinus early T-wave is used as a reference, any changes in the untested QRS complex DIFF(i) from DST(i) is reflective of a systematic departure from the early T-wave template (STDIFF<5), a random signal superimposed on the early T-wave (5<STDIFF<9), or a highly fluctuating signal superimposed on the early T-wave template (STDIFF>9).

The decision rule for QRS classification is stratified according to the noise ratio [(N/S)/(N/S)max] as follows:

$$\text{If } (N/S)/(N/S)\text{ max } \begin{cases} < 1.2, \text{ use table 1} \\ = 1.2 - 2, \text{ use table 2} \\ > 2, \text{ use table 3} \end{cases}$$

where (N/S) max = the maximum value of N/S over 100 normal sinus complexes.

It is apparent that a complex is increasingly more likely to be called noise as the noise ratio increases. In table 1, the limiting values for each classification parameter (T_{rr}, YDEL, and TDIFF) are established on the basis of a histogram limit observed in the normal sinus complex in each decision. In the higher noise ratio decision rule for table 2, the limit values are more restrictive. In this case a 2 percent two-tailed limit value is used for the parameters YDEL and TDIFF. The complex is first tested to determine if it fits the noise criterion. If not, the remaining criteria are examined. In this manner, the noise complexes are prevented from distorting the probability surfaces. A complex is classified as noise if there is a highly fluctuating component superimposed upon the T-wave and if comparison to the early T-wave template does not produce a systematic difference.

The high noise ratio decision rule is shown in table 3. In this case, the decision rule classifies the complex as a ventricular ectopic complex or noise. The complex is a PVC or VEC if the T-wave for the unclassified complex has a systematic discrepancy from a straight line, and if the complex T-wave has a systematic deviation from the normal sinus T-wave template.

Multiform PVC

After the noise rejection criteria have been applied, the frequency of occurrence of the T-wave slope (TDIFF) and the R-R interval for all PVC and VEC are maintained in a two-dimensional frequency of occurrence table. This table represents the probability density functions for all ventricular complexes. The probability table is scanned with the occurrence of each 25 PVC or VEC and

TABLE 1. QRS Classification Criteria in the Case
Where the Noise Ratio Is Less than 1.2

QRS classification	T_{rr}	YDEL	TDIFF
Normal sinus complex	Normal	Normal	Normal
Late VEC	Normal	Abnormal	Abnormal
PVC	Premature	Abnormal	Abnormal
APC	Premature	Normal	Normal

T_{rr} = R-R interval. YDEL = QRS amplitude. TDIFF = Early T-wave slope. VEC = Ventricular ectopic complex. PVC = Premature ventricular complex. APC = Atrial premature complex.

TABLE 2. QRS Classification Criteria in the Case
Where the Noise Ratio Is Between 1.2 and 2

QRS classification	T_{rr}	YDEL	TDIFF	STDIFF	ST_{SEG}
Normal sinus complex	Normal	Normal	Normal	>5	>5
VEC	Normal	Abnormal	Abnormal	<5	<5
PVC	Premature	Abnormal	Abnormal	<5	<5
APC	Premature	Normal	Normal	>5	>5
Noise	N/A	N/A	N/A	>5	>9

N/A = Not applied. STDIFF = Number of zero crossings of D2ST. See table 1 for other abbreviations.

TABLE 3. QRS Classification Criteria in the Case Where the Noise Ratio Is Greater than 2

QRS classification	STDIFF	ST_{SEG}	T_{rr}
Noise	>3	>3	N/A
VEC	<3	<3	Normal
PVC	<3	<3	Premature

See tables 1 and 2 for abbreviations.

regions $R_1, R_2, \ldots R_m$ are determined for up to 10 forms, according to the limit closed curves for each region. The limit for each region is a simply connected closed curve or scroc. The frequency of occurrence of each form is the total number of complexes which are contained within the boundary of each scroc.

The regions $R_1, R_2, \ldots R_m$ are determined sequentially based upon a two-dimensional clustering concept. Region R_1 is determined by scanning the probability density table for the mode frequency point (P_1). Beginning at point P_1, the domain space is expanded in each direction uniformly until a boundary point is established. Boundary points are established as points having zero frequency or a member of some other region on at least one side. The encountered boundary point is entered into the limit scroc table. (The limit scroc table is an encoded parametric array which represents boundary points for the region R_1). The second region R_2 is defined using the maximum frequency of occurrence point P_2 excluding region R_1 and applying the boundary point algorithm on the frequency distribution excluding the region R_1. This process is sequentially repeated until all nonzero frequency of occurrences are exhausted. Since some regions may adjoin, the limit values for these regions are determined using a Bayesian decision rule as outlined by Hansmann (7). The major difference is in the density function and the region definition. In this case the frequency distribution for the i-th region is taken directly from the frequency of occurrence table for the appropriate region R_i. Examples of multiform PVC recognition are shown in figures 3 and 4.

RESULTS

The accuracy of our algorithms for noise rejection and multiform ventricular ectopic identification was tested in 24-hour "high noise" Holter recordings in 25 ambulatory patients with a moderate to high incidence of VEC. Of the 25 patients, 5 had uniform PVC, 9 had VEC, 11 had multiform PVC and/or VEC, and 6 had atrial premature complexes. After processing via the noise algorithm, the computer correctly identified the presence of the multiform ventricular ectopic

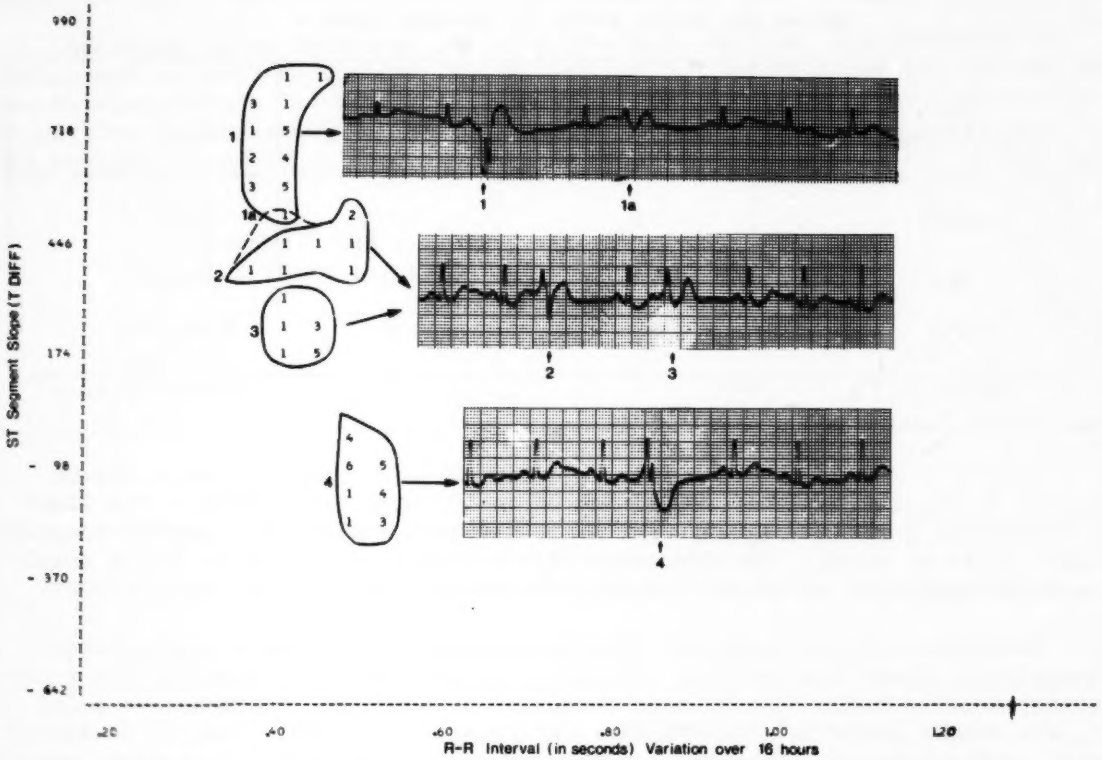

FIGURE 3. Two-dimensional histogram of R-R interval and ST segment slope for premature ventricular complex (PVC) and ventricular ectopic complex (VEC). The frequencies of occurrence of all PVC's in a typical ambulatory ECG recording are indicated by the number enclosed in scrocs. Each form is identified and an example ECG strip is shown. In this case the Bayesian decision rule was applied to define the boundary between forms 1 and 2. The temporal nature of these boundaries is indicated with the form labeled la and the dashed line. The form labeled la was first classified as form 2 and finally reclassified to form 1.

complex in all 11 patients. The five patients who had uniform PVC were correctly identified as having only one form. Application of the noise algorithm reduced false positive PVC identification in these high noise tapes to 2.3 percent and false negative to 3.9 percent, representing 0.04 percent false positive and 0.06 percent false negative classifications of the total number of complexes.

DISCUSSION

We believe that progress has been made in reducing errors in PVC and VEC identification due to baseline shift and muscle noise and that an automated system without the need for extensive editing and reprocessing is becoming feasible. While editing will certainly remain a vital component of any system

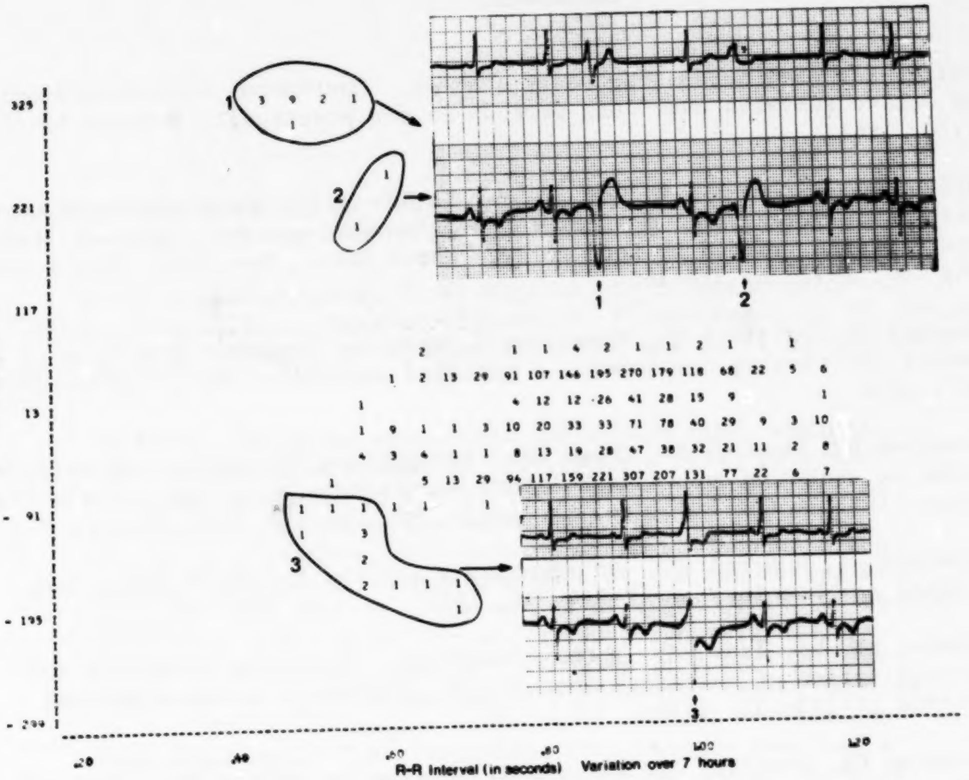

FIGURE 4. Two-dimensional histogram of R-R interval and ST segment slope parameters for PVC's, VEC's, and limit values for decision surface normal sinus complexes. The three forms were identified by the computer. The computer monitored channel 2. The limiting values of the decision surface shows the overlap of the decision surface and the scrocs for each PVC.

claiming quantitative capability, it can be excessively cumbersome in high noise tapes, and is somewhat dependent upon the skill and patience of the editor. In addition, unless almost one-to-one review is done, there is no control over false negative VEC identification. Perhaps more importantly, if a system is being utilized to assess heart rate or QT alterations which may presage VEC emergence, the significance of such events may be obscured or enhanced by the inclusion of false positive or negative PVC or VEC in the statistical analyses.

The clustering technique described in this paper differs significantly from other techniques in that the definition of the region for a given form is based upon the frequency of occurrence of events within the region. In cases where there are two forms sufficiently close together to require definition of a boundary between them, the decision on boundary location is based upon the expected value of the frequency distribution as expressed by a linear extrapolation to zero frequency of occurrence in all directions. While this technique requires additional logic and is more complicated than other techniques, it is felt that the added calculations provide flexibility not present in other algorithms.

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ACUTE MYOCARDIAL INFARCTION REGISTER: FEASIBILITY OF IMPROVING DIAGNOSES AND CLARIFYING THE NATURE OF SUDDEN DEATH

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ABSTRACT. An acute myocardial infarction (AMI) register was introduced in Kaunas in 1969. Since 1971, this work has been conducted in coordination with other centers in Europe, and following the recommendations of the World Health Organization (WHO) Working Group on Ischaemic Heart Disease Registers. Cumulative data of the Kaunas register for 1969-1978 show that the number of cases of AMI increases every year. The morbidity per 1,000 persons in all age groups increases in parallel, reaching 1.66 in 1978. Because of the high percentage of prehospital deaths from AMI in the city of Kaunas, a prehospital medical service has been organized. These organizational changes have had a favorable effect. From 1972 to 1978, the number dying of AMI before reaching the hospital decreased from 64.9 percent to 40.7 percent. Hospital lethality, however, increased from 21.0 percent to 22.5 percent. This can be explained by the more rapid admission of AMI patients to the hospital. Total lethality decreased from 44.0 percent to 33.9 percent. Analysis of the morbidity per 1,000 inhabitants of Kaunas, classified by age and sex of AMI patients, showed that morbidity from AMI increased among younger persons, regardless of sex, and especially in persons between the ages of 45 to 54; morbidity decreased among the elderly. Overall results indicate a high morbidity and mortality from AMI, especially among the older age groups. Introduction of the AMI register has led to a reduction of total mortality from AMI in Kaunas and has prompted a directed search for means of controlling AMI.

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INTRODUCTION

The acute myocardial infarction register is an information system that provides the basis for elaboration of a strategy and tactics for a national health service program to combat coronary (ischemic) heart disease. Making right decisions and choosing effective measures primarily depend upon the reliability and accuracy of registry data on the incidence of disease, and the particular features of its course and outcomes.

The methodology used by the AMI register has been adapted to meet the requirements of large-scale studies. Nevertheless, a certain number of AMI cases are not diagnosed clinically because of problems in diagnosis (1,2). These same difficulties were also encountered in the WHO European Regional Register on AMI. For this reason, the following diagnostic categories have been introduced for acute myocardial infarction: "definite," "possible," and "no signs." Whereas the diagnostic criteria for "definite" myocardial infarction are clear-cut, those for "possible" myocardial infarction are extremely diffuse. Moreover, in cases of sudden death, no emphasis is placed on the need for morphological examination of acute ischemic lesions of the myocardium occurring within the last 12 hours (3); that is, the very first stages in the development of AMI are overlooked. This results in assigning to the "possible" AMI group cases in which the actual condition is not fully elucidated.

The objective of our study was to define more precisely the clinical and morphological criteria for diagnosis of AMI, including instances of sudden death, and, in this way, to move closer toward a more definitive understanding of "possible" myocardial infarction.

MATERIALS AND METHODS

Kaunas is a city in the northwestern part of the USSR with a population of about 360,000. It has a 140-bed specialized cardiology clinic and hospital with a 12-bed coronary care unit. All persons suspected of having AMI or unstable angina are hospitalized. Ninety percent of all calls are handled by four specialized cardiology mobile units. Each team is headed by a cardiologist. The average time from receiving a call by the emergency service to the physician's arrival at the patient's side is 11.3 minutes.

The AMI register program was started in Kaunas in 1969. Since 1971, we have followed the procedures recommended by the WHO Working Groups on Ischaemic Heart Disease Registers (4,5). All inhabitants of Kaunas, regardless of the place of illness or treatment, are included in the program. Register physicians query patients using a standard questionnaire which we developed based on the well-known Rose questionnaire, and are guided by WHO criteria in establishing diagnoses. In addition, the following records are scrutinized: in-hospital and polyclinic medical histories of the disease, patient charts obtained from the hospital, autopsy reports, written forensic statements, and death certificates.

All patients who die in hospitals are autopsied. The percentage of autopsies performed on males up to 65 years of age, who died in the prehospital stage, is 89 percent; the percentage for all age groups of both sexes is 82.3 percent. A special, in-depth, morphological study of the coronary arteries and the myocardium was conducted in 76.7 percent of the cases of sudden death (i.e., death occurring within 6 hours from the onset of acute terminal cardiac arrest). Transverse sections, at 2-3 mm intervals, are used to study the localization, degree, extent, and nature of obstructions in the four subepicardial coronary arteries. Segments showing occlusions of 50 percent or more stenosis are subjected to a step-by-step, serial histological examination. The nature, localization, and volume percentage of focal damage to the total volume of the left ventricle and interventricular septum of the heart are evaluated by macroscopic study of transverse sections at 0.5-1 cm intervals, as well as histologically (histotopograms).

This paper presents data on the in-depth morphological study of the condition of the myocardiums of 85 hearts from males 31-69 years of age who died suddenly outside of the hospital, and who were listed in the register as suspected AMI. Following the criteria in the register, 59 were listed as "possible," and 26 as "definite" infarctions.

RESULTS AND DISCUSSION

AMI Register Data

Data from the AMI register for 1972 and 1978 were compared (table 1) to ascertain the trends in morbidity and lethality. The number of cases of AMI recorded during this period increased. The morbidity per 1,000 increased from 1.39 in 1972 to 1.66 ($p < 0.01$).

We regard the increase in morbidity as the result of improved diagnostic procedures [introduction into clinical practice of 36-lead electrocardiogram (ECG) recordings, more detailed morphological examination, etc.] and improved registration of the disease, particularly in cases without a clear clinical

TABLE 1. Comparative Data From the Acute Myocardial Infarction Register in Kaunas in 1972 and 1978

Year	Morbidity per 1,000	Out-of-hospital deaths (%)	In-hospital deaths (%)	In-hospital lethality (%)	Total lethality (%)
1972	1.39	64.9	35.0	21.0	44.0
1978	1.66	40.7	59.3	22.5	33.9
p	< 0.01	< 0.001	< 0.001	> 0.5	< 0.001

pattern. Expansion of indications for hospitalization of patients with suspected AMI or unstable angina pectoris, particularly in specialized cardiology units, has contributed to the finding of new cases of the disease, and has influenced the trends in morbidity. Improvement in prehospital medical service and more rapid hospitalization of patients with a suspected preinfarction condition have resulted in a considerable change in the distribution of fatal outcomes between the prehospital and hospital stages. The percentage of out-of-hospital deaths (lethality) (of all patients who died of AMI) fell from 64.9 percent in 1972 to 40.7 percent in 1978 ($p < 0.001$). No significant change occurred, however, in hospital lethality which was 21.0 percent in 1972 and 22.5 percent in 1978. It should be noted that the percentage of total lethality (hospital and out-of-hospital deaths for all registered AMI cases) decreased from 44.0 percent in 1972 to 33.9 percent in 1978 ($p < 0.001$). This can be attributed to improved medical care of AMI patients at all stages of the disease.

Morbidity per 1,000, by age and sex, was higher among men aged 20-64 years and 65-80 years than it was among women (table 2). Morbidity among persons 20 to 64 years of age of both sexes was 1.3 in 1977, i.e., higher than in 1972 ($p < 0.01$).

TABLE 2. Morbidity of Acute Myocardial Infarction in Kaunas by Sex and Age Per 1,000

Sex	Year	Age (years)	
		20-64	65-80
Males	1972	1.6	14.0
	1977	2.3	13.3
	p	< 0.01	> 0.5
Females	1972	0.3	7.3
	1977	0.4	6.5
	p	> 0.5	> 0.5
Total	1972	0.9	9.5
	1977	1.3	8.8
	p	< 0.01	> 0.5

The mortality per 100,000, by age and sex, was also higher among men than among women in the corresponding age groups (table 3). The mortality among both sexes in the 65-80 year age group was greater by a factor of 11 in 1972, and by a factor of 8 in 1977, than it was for those in the 20-64 age group.

Lethality in both age groups decreased in 1977 (table 4). In the group 20-64 years old, lethality decreased from 42.2 percent in 1972 to 29.2 percent in 1977 for both sexes.

As noted earlier, recorded morbidity for AMI depends upon the use of standardized definitions and specific criteria. But, as data provided by individual authors working with the WHO methodology indicate, the number of "possible" acute myocardial infarctions varies from 13 percent to 48 percent (6,7). This discrepancy stems, in particular, from the many variants in disease symptoms and from differences in interpretation of diagnostic criteria in individual clinical cases.

Clinical Data

Analysis of the anamnestic data on 258 patients with "definite" and "possible" AMI revealed that the incidence of intermittent claudication, previous

TABLE 3. Mortality From Acute Myocardial Infarction in Kaunas by Sex and Age Per 100,000

Sex	Year	Age (years)	
		20-64	65-80
Males	1972	69.3	632.6
	1977	73.1	544.3
	p	> 0.5	> 0.5
Females	1972	12.3	332.9
	1977	6.8	199.4
	p	> 0.5	< 0.01
Total	1972	39.3	432.6
	1977	38.2	315.1
	p	> 0.5	> 0.5

TABLE 4. Lethality of Acute Myocardial Infarction Distributed by Sex and Age

		Age (years)	
Sex	Year	20-64	65-80
Males	1972	43.4	45.3
	1977	32.2	40.8
	p	< 0.01	> 0.5
Females	1972	37.1	45.5
	1977	15.4	30.6
	p	< 0.01	< 0.01
Total	1972	42.2	45.4
	1977	29.2	35.8
	p	< 0.01	< 0.01

myocardial infarction, typical angina pectoris, and hypertension was not significantly different between these categories (table 5).

The WHO criteria assign particular importance to the pain syndrome in diagnosis of AMI. We analyzed the frequency of occurrence of the pain syndrome and its individual symptoms. As table 6 shows, the presence of pain in the chest among patients with "definite" myocardial infarction was established in 86.5 percent of the cases. Pain with widespread radiation, pain lasting longer than 20 minutes, and severe pain were noted equally often among patients with "definite" and "possible" myocardial infarction. However, we noted a combination of all four symptoms in only 53 percent of the patients with "definite" myocardial infarction, whereas a combination of severe pain, localization in the chest, and pain lasting longer than 20 minutes was noted in 67.3 percent of the patients with "definite" myocardial infarction. Thus, even if symptoms of the pain syndrome are observed, "possible" myocardial infarction cannot be excluded.

Analysis of the diagnostic value of a combination of criteria in 230 cases of "definite" AMI revealed that 78.3 percent of the patients had typical pain, 73.5 percent had a clear-cut increase in serous enzyme activity,

TABLE 5. Medical Anamnestic Data Prior to Acute Myocardial Infarction

	Myocardial infarction			
	"Definite"		"Possible"	
Anamnestic data	No.	% ± S.D.	No.	% ± S.D.
Myocardial infarction	60	26.1 ± 2.6	8	28.6 ± 5.8
Angina pectoris	104	45.2 ± 3.3	13	46.4 ± 9.4
Intermittent claudication	32	13.9 ± 2.3	5	17.9 ± 7.2
Hypertension	57	24.8 ± 2.8	9	32.1 ± 8.8

S.D. = Standard deviation.

TABLE 6. Characteristics of the Pain Syndrome in Patients With Acute Myocardial Infarction

	Myocardial infarction			
	"Definite"		"Possible"	
Pain symptoms	No.	% ± S.D.	No.	% ± S.D.
In chest	199	86.5 ± 2.3	28	100.0 ± 0
Widespread radiation	153	66.5 ± 3.1	18	64.3 ± 9.1
Longer than 20 minutes	183	79.6 ± 2.7	24	85.7 ± 6.6
Severe	178	77.4 ± 2.8	20	71.4 ± 8.5

S.D. = Standard deviation.

and 91.7 percent had definite ECG changes (figure 1). Nevertheless, only 50.4 percent of the patients with "definite" AMI were found to have this combination of typical pain, definite hyperfermentemia, and definite ECG changes. Pedoe (8) cites similar data. "Possible" myocardial infarction was accompanied by typical pain, questionable ECG shifts, and a questionable increase in enzyme activity in 41.4 percent of the patients, whereas typical pain, questionable ECG shifts, and normal enzyme levels were present in 58.6 percent of the patients.

Morphological Data

In-depth morphological examination of all 85 sudden out-of-hospital deaths revealed obstructive damage (atherosclerotic stenosis and thrombotic occlusion) in at least one of the four main coronary arteries. Obstruction of the lumen of the artery was severe (75 percent and more) in 91.8 percent of the cases, moderate (50-75 percent) in 7.1 percent, and slight (less than 50 percent) in just one case (1.1 percent) (table 7). Severe and moderate obstruction most often existed simultaneously in the other arteries. If severe obstruction was present in one or two main arteries, moderate atherosclerotic stenosis was found in the others. Obstructive damage, as most

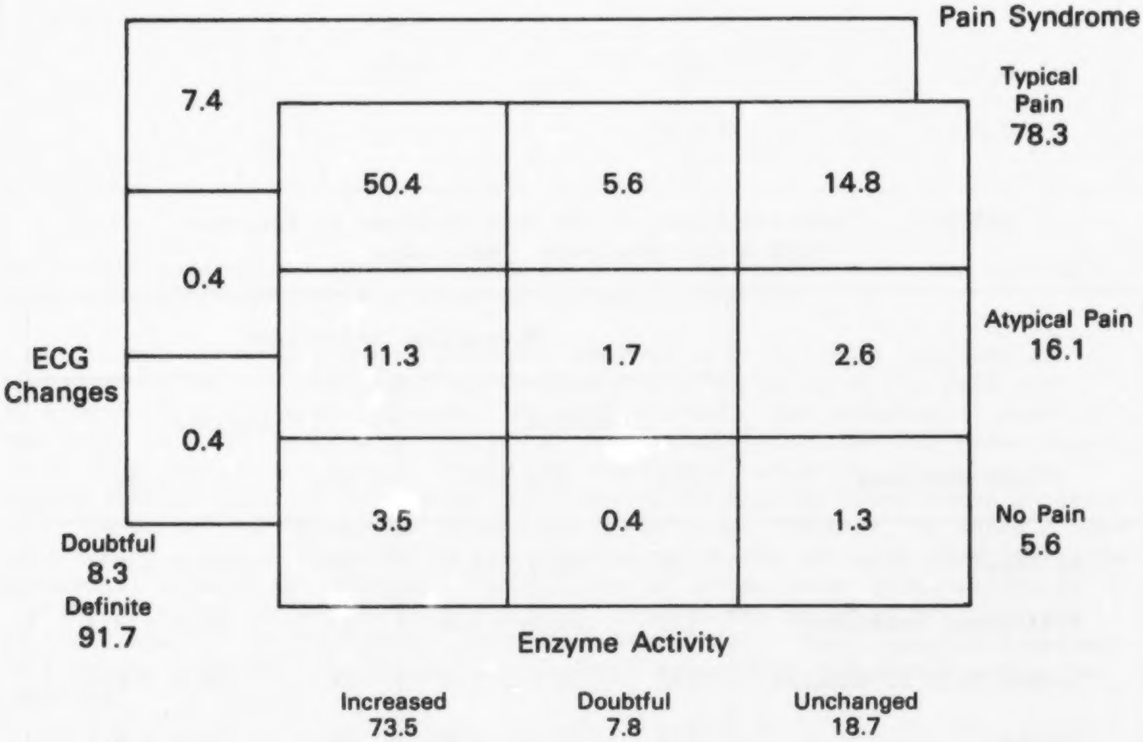
Pain Syndrome
Typical Pain 78.3
Atypical Pain 16.1
No Pain 5.6
ECG Changes
Doubtful 8.3
Definite 91.7
7.4
0.4
0.4
50.4
5.6
14.8
11.3
1.7
2.6
3.5
0.4
1.3
Enzyme Activity
Increased 73.5
Doubtful 7.8
Unchanged 18.7

FIGURE 1. Frequency of combination of diagnostic criteria (in %) in patients with "definite" acute myocardial infarction.

TABLE 7. Characteristics of Obstructive Damage to Coronary Arteries in Sudden Death Cases With "Possible" and "Definite" Myocardial Infarction

Category of infarction	No. cases	Obstructive damage (atherosclerotic stenosis and thrombotic occlusions)					
		< 50%		50-75%		≥ 75%	
		No.	%	No.	%	No.	%
"Possible"	59	1	1.7	4	6.8	54	91.5
"Definite"	26	-	-	2	7.7	24	92.3
Total	85	1	1.1	6	7.1	78	91.8

researchers point out (9-12), is most often localized in the proximal and medial segments of the left anterior descending and right coronary arteries. Combined obstructive damage to several coronary arteries simultaneously is most often localized in these arteries.

Thrombotic occlusions (old and recent) were discovered in 78 cases (92 percent): old occlusions were found in 52 percent and recent occlusions were found in 84 percent of all those observed. A combination of organized and recent thrombi, established in 52 percent of the cases of acute thrombosis, was of a dual nature: (a) the remaining lumen and/or channels of the recanalized thrombus were recently thrombosed, and (b) the thrombus was localized in another artery. The old, as well as recent, thrombotic occlusions were most often localized in the right coronary artery, although a recent thrombus was discovered in the left anterior descending artery twice as frequently as old organized thrombi. No correlation was found between the incidence of a recent thrombosis on the duration of the terminal event. No significant differences were found in any of the characteristics of obstructive damage to the coronary arteries in cases of "possible" and "definite" infarctions described above, other than a tendency toward an increase in the frequency ($p < 0.05$) of acute thrombosis in the "definite" infarction group (78 percent and 96 percent, respectively). Like other authors (13-15), we noted a step-by-step development in the acute pathology of the subepicardial coronary arteries: ulceration and ruptures of the atheromatous patches with subsequent formation of parietal (thrombocytic and fibrinous) and obstructive thrombi.

Foci of substitutional myocardial fibrosis were found in 88.2 percent of the cases, with postinfarction scars (foci with a diameter of 0.5 cm and greater) in 73.3 percent, and multifocal fibrosis in 26.7 percent of the cases.

The latter were observed in more than one-half of the cases by only studying histotopograms of the myocardium. There was complete agreement among the characteristics of substitutional myocardial fibrosis for the "definite" and "possible" categories of myocardial infarction (table 8).

Acute focal lesions were observed in all 85 cases upon careful histological examination of all parts of the heart. Histological criteria for morphological diagnosis of AMI proposed by a WHO cooperative study (16), and the experience of some authors (17), distinguish two stages in the development of acute myocardial infarction:

- Early myocardial infarction when there is no defined focus of coagulative necrosis visible to the naked eye

- Definite myocardial infarction with macroscopically detected necrosis.

Early myocardial infarction predominated among the cases registered as "possible," while definite myocardial infarction included almost all cases in the "definite" infarction group (table 9). Interestingly, no significant differences existed in the duration of the terminal acute cardiac arrest in individuals who died in the early stage of infarction development compared with those already having a necrotic focus, that is, between those with "possible" and "definite" infarctions. Prodromal symptoms, as well as a medical

TABLE 8. Characteristics of Substitutional Myocardial Fibrosis in Sudden Death Cases With "Possible" and "Definite" Myocardial Infarction

Category of infarction	No. cases	Substitutional myocardial fibrosis							
		Total		Postinfarction scars				Multifocal fibrosis	
				Transmural		Nontransmural			
		No.	%	No.	%	No.	%	No.	%
"Possible"	59	52	88.1	28	53.8	8	15.4	16	30.8
"Definite"	26	23	88.4	14	60.8	5	21.8	4	17.4
Total	85	75	88.2	42	56.0	13	17.3	20	26.7

TABLE 9. Characteristics of Periods of Recent Myocardial Infarction in Sudden Death Cases With "Possible" and "Definite" Myocardial Infarction

Category of infarction	No. cases	Stage of infarction development			
		Early		Definite	
		No.	%	No.	%
"Possible"	59	43	72.9	16	27.1
"Definite"	26	2	7.7	24	92.3
Total	85	45	52.9	40	47.1

history of ischemic heart disease, were found with identical frequency in these two groups.

Thus, sudden death, included in the register under the "definite" and "possible" categories of myocardial infarction, occurred in the overwhelming majority of cases against a background of an almost identical and distinctive morphological pattern of coronary (ischemic) heart disease. The acute fatal episode was associated with acute focal damage to the myocardium, that is, myocardial infarction in the early and definite periods of its development.

SUMMARY

Clinical and morphological data on the feasibility of using the AMI register to explain the patterns in the development, course, and outcome of acute myocardial infarction are presented.

An in-depth, specialized morphological study of the coronary arteries and the myocardium is the basis for concluding that sudden death occurs against a background of distinct morphological coronary (ischemic) heart disease in the overwhelming majority of cases, that is, with chronic obstruction of the lumen (atherosclerotic stenosis and old thrombotic occlusion) of 50 percent and more in at least one of the main coronary arteries, and with foci of substitutional fibrosis present in the myocardium. The acute terminal episode is associated with the occurrence of acute pathology in the subepicardial coronary arteries in the form of ulcerations, rupture of the atherosclerotic plaques, and subsequent formation of microthromboli and parietal and

obstructive thromboses explaining the development of acute focal myocardial damage. Such damage can, in accordance with the histological criteria of AMI, be qualified as early and definite recent infarctions, that is, the transitional stages in the development of the disease process.

Identical anamnestic data and the compatible clinical course of "definite" and "possible" AMI make it possible to consider that cases of sudden death in established "possible" infarction, in fact, are cases of death that had their onset in the early or earliest stages of myocardial infarction. Macroscopic, as well as histological, indices of the diagnosis must be included among the diagnostic criteria for the register of AMI in order to obtain an accurate explanation of these deaths.

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LONG-TERM TRENDS IN THE INCIDENCE OF ISCHEMIC HEART DISEASE

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ABSTRACT. Premature sudden death is part of the larger problem of ischemic heart disease (IHD). Although much is known about the causes of IHD it is still not clear why the death rates from IHD show rises and falls over periods of years or decades. Much discussion of the recent downtrend in IHD mortality in the U.S. concerns whether this downtrend is primarily due to prevention or treatment. A clear demonstration of a parallel downtrend in incidence would support the role of prevention. Data from the Kaiser-Permanente Medical Care Program in northern California during the years 1971-1977 show a 27 percent drop in rates of hospitalization for acute myocardial infarction (MI), an important component of IHD incidence. Total IHD declined 18 percent. Trends in risk factors have not yet been determined but data are available for subscribers who have had repeated multiphasic health checkups. A comparison of time trends in IHD incidence and in risk factor levels in different countries would help explain these trends. This could be an area for fruitful U.S.-U.S.S.R. collaborative study.

INTRODUCTION

Premature sudden death is one of the most serious consequences of ischemic heart disease. Most premature nontraumatic sudden death in our societies is due to IHD. While much important work in preventing premature sudden death is

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and must be directed toward persons who already have IHD, the solution to the sudden death problem will also come largely from the prevention of IHD.

Although much is known about factors predisposing to IHD, there are still many unanswered questions. One of the most tantalizing questions of recent concern pertains to the long-term upswings and downswings in the occurrence of IHD. To what extent do living habits play a role in these trends? How much does improved treatment contribute to a downtrend in mortality? Could there be a cause of IHD totally different from the recognized risk factors, such as perhaps an infectious agent, that causes epidemics of IHD?

Long-term trends of IHD in the United States have provoked considerable discussion recently. There was an increase in the IHD death rate into the mid-1960's and then a fall amounting to over 20 percent. The National Heart, Lung, and Blood Institute sponsored a large conference concerning the recent decline in IHD mortality (1). Experts in many aspects of IHD assembled and contributed their views. Each had his or her own belief as to why the downtrend is occurring. Assembling this divergent group provided a valuable educational experience by helping to dispel "the blind men and the elephant" problem. According to the fable, several blind men assembled around an elephant for the purpose of characterizing it. One got hold of its tail and said it was like a snake. Another felt one of its legs and said it was like a tree, and so on. Each had a limited "view" of the elephant. When the experts assembled and shared their perspectives on the IHD problem it became clear to all that the decline in IHD mortality was probably due to a combination of factors such as changes in diet, cigarette smoking, exercise, more and better treatment of hypertension, and better outpatient and hospital care, both medical and surgical.

One of the important questions that has not yet been answered well concerns the extent to which the recent decline in IHD mortality (a 20.7 percent decline in the U.S. from 1968 to 1976) reflects a decline in IHD incidence. If the incidence is decreasing, this would suggest that lifestyle changes and control of risk factors are important. If the incidence is staying the same, then the decline in mortality must be due to improved treatment. To help answer this question we assembled data concerning hospitalizations of subscribers to the large Kaiser-Permanente Medical Care Program, in the northern California region.

METHODS

Computerized hospitalization records have been prepared by the program each year since 1971. From June 1971 to June 1977, the midyear subscriber population grew from 1.00 to 1.38 million persons. In the middle year, 1974, the membership numbered 1.19 million, or one of every 178 persons in the total U.S. population. The June 1974 membership aged 35-84, totaling 430,270 persons (50 percent female), was used as the standard population for direct age-sex adjustment of rates. The subscribers and their families in this prepaid medical care program are a heterogeneous group who join largely as members of employment groups such as teachers, industrial and trade unions, and civil servants. Persons can join individually if they pass a medical examination. All ethnic and socioeconomic groups are included, but it is believed that persons at the extreme high and low ends of the socioeconomic spectrum may be underrepresented. About 90 percent or more of the hospitalizations of subscribers are at Kaiser Foundation Hospitals.

Hospital record room personnel in the 13 Kaiser Foundation Hospitals in the region coded the discharge diagnoses according to the Eighth Revision of the International Classification of Diseases, Adapted (ICDA). For the present analysis, only the first three "primary" diagnoses in each record were scanned. The rates presented for each year refer to the number of persons hospitalized at least once (not the number of hospitalizations) among those aged 35-84. The diagnostic codes of interest were 410--acute myocardial infarction, and 410-414--all coronary heart disease (CHD). Racial identification is not routinely available in health plan records so race-specific rates could not be tabulated.

RESULTS

The proportion of persons hospitalized each year for acute MI dropped in a fairly steady fashion from 4.05 per 1,000 in 1971 to 2.96 per 1,000 in 1977, a 27 percent decline. The age- and sex-specific proportions for each decade were examined (figures 1 and 2). Men aged 45-54 and 55-64 and women aged 55-64 and 65-74 showed fairly similar smooth downtrends. Men aged 65-74 and 75-84 and women aged 45-54 and 75-84 showed less regularity, possibly due to smaller numbers of subjects, but all showed a net improvement from 1971 to 1977. Men aged 35-44 showed a slight rise to 1973 and 1974 and a subsequent fall with virtually no net change from 1971 to 1977. The number of women aged 35-44 with MI was very small and the proportion showed almost no change from 1971 to 1977.

The proportion hospitalized for any CHD also showed a fairly steady decline from 1971 to 1977--10.81 per 1,000 to 8.90 per 1,000, or a drop of 18 percent. Persons in the 35-84 age group hospitalized for any reason showed a rather steady decrease, from 77.27 per 1,000 to 67.61 per 1,000, a 13 percent decline.

For each of the diagnostic categories, we calculated the proportions dying in the hospital of all persons hospitalized each year. These may be regarded as approximating case fatality rates. For acute myocardial infarction, this proportion varied between 12.9 and 15.9 percent with no clear trend through the years studied. For all CHD, the fraction fell from 11.8 percent to 9.6 percent between 1971 and 1972 with virtually no trend thereafter. A small, slightly irregular downtrend was also observed for all hospitalized persons--from 6.2 percent in 1971 to 5.1 percent in 1977.

To help determine whether the trend in admissions for MI involved new or recurrent events or both, we reviewed a sample of records of men aged 55-64 with myocardial infarction in 1971 and 1977 (the 7-year drop in hospitalization rate for MI in this age-sex group was 27 percent). All available hospital records for two facilities (Oakland and San Francisco) were reviewed

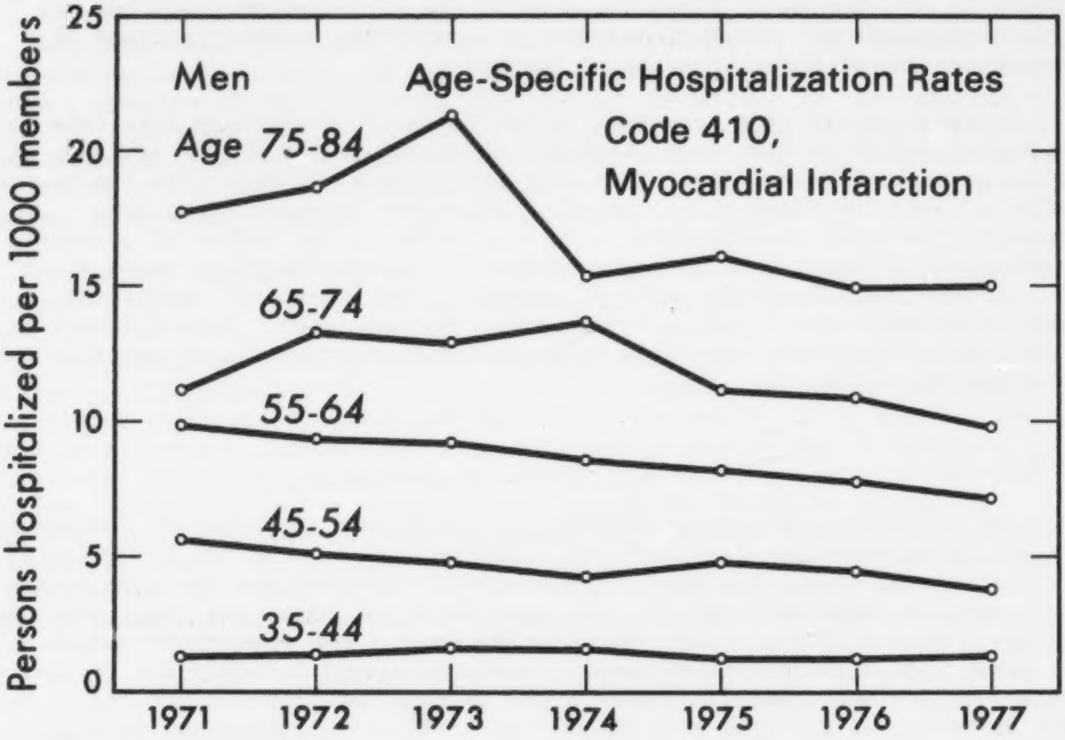

FIGURE 1. Age-specific proportions of male subscribers, aged 35-84, hospitalized for acute myocardial infarction (ICDA code 410). Kaiser-Permanente Medical Care Program, northern California region, 1971-1977.

to determine whether each man's first infarction in that year was the first in his life or whether prior infarctions had been recorded. A total of 189 case records were reviewed, representing 90 percent of the 210 hospitalizations of interest in the two facilities. In 1971, 82 of 120 myocardial infarctions (or 68 percent) were first events. In 1977, 44 of 69 (or 64 percent) were first events. The similiarity in the two percentages indicates that both new and recurrent infarctions participated in the downtrend.

We also tried to determine whether the decline might be an artifact due to a change in membership composition over the years, with new members possibly being derived from lower-risk segments of the population. Age- and sex-adjusted proportions hospitalized for MI in 1971 and 1977 were calculated for that portion of the membership who joined the health plan before 1969. The results were very similar to the findings for all subscribers--3.91 per 1,000 in 1971 and 2.87 per 1,000 in 1977, again a 27 percent drop.

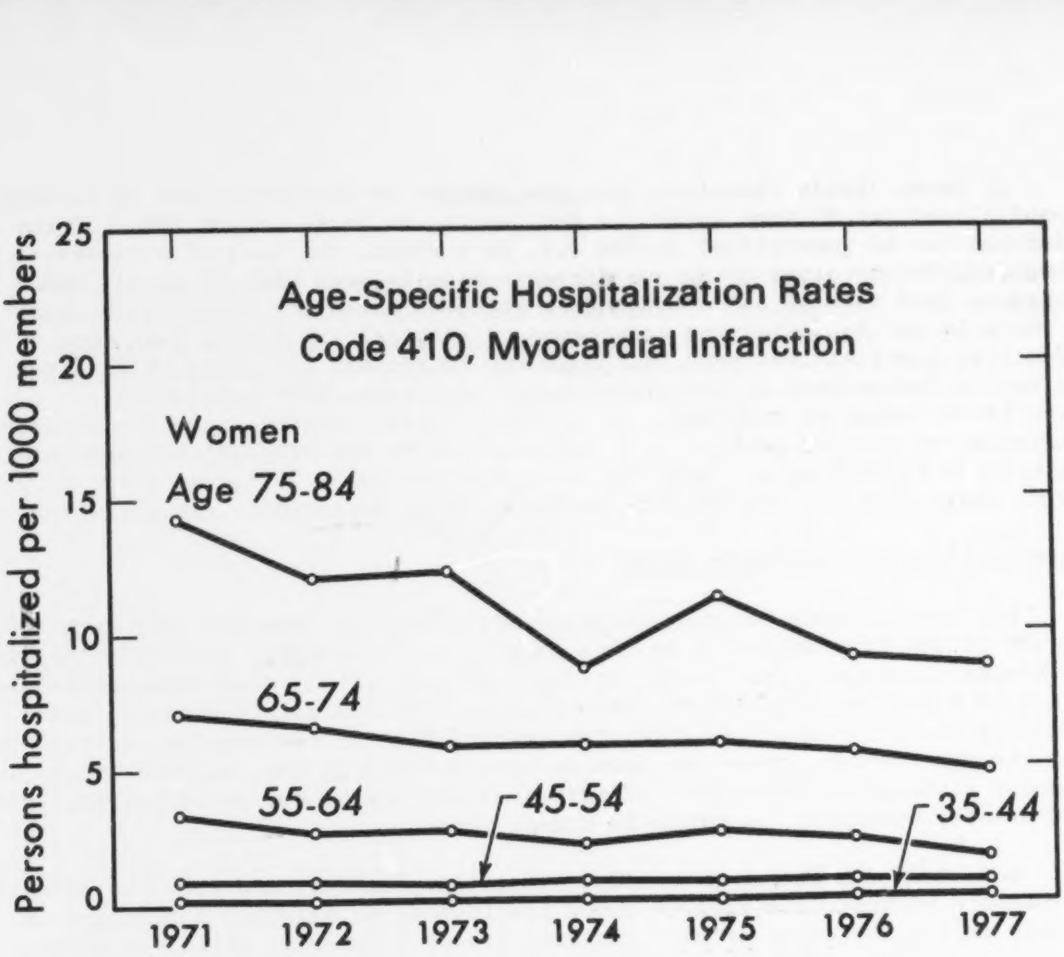

FIGURE 2. Age-specific proportions of female subscribers, aged 35-84, hospitalized for acute myocardial infarction (ICDA code 410). Kaiser-Permanente Medical Care Program, northern California region, 1971-1977.

COMMENT

The most meaningful of the data presented are probably those pertaining to acute MI. This is usually a discrete dramatic event in the course of CHD with objective criteria for diagnosis. Discussion with local cardiologists revealed no reason to believe that the criteria for admission or final diagnosis of this condition would have systematically changed in the program over the 7-year period. The concomitant decline in the proportion of persons admitted for any CHD or for all conditions shows that the diagnosis and coding of acute MI were not shifting to other categories. Unfortunately, these data for MI do not provide the ideal measure needed--that is, the incidence of new clinical CHD. Acute MI is only one component of the clinical spectrum of CHD and our hospitalization data would include both new and recurrent events and would lack unrecognized infarctions. Review of a sample of hospital records indicated, however, that new infarctions were participating in the decline at least as much as were recurrences.

It seems likely therefore that the decline in the proportion of persons hospitalized for MI does reflect a decline in the incidence of CHD. If our findings can be generalized to the U.S. as a whole, the lack of a clear-cut downtrend in mortality among our patients hospitalized with MI or all CHD suggests that most of the downtrend in national mortality rates in the past 7 years is due to decreasing occurrence of clinical CHD rather than more effective hospital treatment, although the latter may certainly be a factor, as may be improvement in outpatient care. Our subscriber population's experience cannot be attributed to coronary bypass surgery since the procedure, performed at outside institutions and paid for by the program, has been employed relatively infrequently. Data are available for 1975, 1976, and 1977, during which there were 23, 54, and 125 coronary bypass operations, respectively.

Potential for Collaborative Study

One area of possible collaboration with the USSR concerns long-term trends in IHD of the type discussed in this paper. The Myocardial Infarction Registry in Kaunas, Lithuania, has carefully surveyed myocardial infarction and sudden death in a defined population. As a byproduct of its main functions, the Registry should produce valuable time trend data. It has been shown that, during the past decade, there has been an upward trend in the incidence of MI in Kaunas, although at least part of this increase may be due to better reporting and greater community awareness in recent years.

Data from the Kaunas Myocardial Infarction Registry and from the Kaiser-Permanente Medical Care Program could continue to be examined for long-term trends in the incidence of MI. The data are of a somewhat different scope since the readily available Kaiser-Permanente data cover hospitalizations whereas the Kaunas Registry is community-based. If different time trends of incidence can be established it may be possible to compare trends in risk factor levels and other characteristics in the two populations in an attempt to identify which factors explain the incidence trends.

The assistance of Donna Wells and Beverly Whitmarsh is gratefully acknowledged.

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PREDICTION OF VENTRICULAR ARRHYTHMIAS DURING RECOVERY FROM MYOCARDIAL INFARCTION

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ABSTRACT. Complex ventricular arrhythmias occurring in the first year after myocardial infarction (MI) have been associated with an increased risk of sudden death. Several studies have shown that these arrhythmias are most likely to occur in patients with depressed left ventricular function and/or extensive MI. Moreover, these arrhythmias in the setting of poor ventricular function imply an especially poor prognosis. Peak premature ventricular complex (PVC) rates and runs of ventricular tachycardia were examined in a prospective study of more than 200 survivors of MI using repetitive, monthly long-term electrocardiographic (ECG) recordings and the Argus/H system to quantitate ventricular arrhythmias. These arrhythmias were correlated with clinical data obtained during the acute infarction. The PVC rates were related to heart size, site of infarction, and peak level of serum glutamic oxaloacetic transaminase (SGOT). Highest PVC rates were found in 18 patients with anterior infarcts, SGOT > 240, and cardiomegaly. Lowest PVC rates were found in 17 patients with anterior infarcts, no cardiomegaly, and SGOT < 120. The difference in PVC rates in these two groups was greater than 300-fold. Intermediate PVC rates were found in all patients with inferior infarcts and anterior infarcts with SGOT between 120-240. Ventricular runs occurred in 30 percent of patients with repeated monitoring and were also related to clinical data obtained during the acute infarction. Patients with runs had higher peak SGOT levels, a higher incidence of congestive heart failure, cardiomegaly, left

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ventricular hypertrophy (LVH), and more frequent atrial premature complexes (APC's), PVC's > 6/minute, and intraventricular conduction defects (IVCD) during acute infarction. A multivariate logistic model was developed which successfully allowed us to place patients into quartiles of risk (4 percent in the lowest quartile and 49 percent in the highest). These studies show that survivors of acute infarction can be stratified into groups with markedly different PVC rates and incidence of runs. These subgroups may be especially suitable for clinical trials of antiarrhythmic agents.

INTRODUCTION

Ventricular arrhythmias commonly occur in the posthospital phase of acute MI (1,2). These arrhythmias have been related to indices of left ventricular function and have also been associated with an increased risk of sudden death in the convalescent period (2,3). Several studies have found that patients with complex ventricular arrhythmias including ventricular tachycardia, multifocal PVC's, bigeminy, and couplets, as well as high frequency PVC rates have an increased mortality (2-6). However, these studies have differed significantly in patient population studied, the duration of long-term ECG monitoring, the frequency of recordings, the time periods after infarction during which the recordings were performed, and the precision of ventricular arrhythmia detection. These various studies have attempted to define subgroups of patients with coronary artery disease who are subject to differing risks of sudden death and/or ventricular arrhythmias.

This paper reports on two aspects of ectopic ventricular activity--PVC frequency and the presence of runs of ventricular ectopic activity (VEA)--in a well-defined group of patients who survived acute MI. These arrhythmias have been related to certain easily made clinical observations during the acute infarction. We have obtained repeated long-term ECG recordings in this population starting 2 weeks after infarction, and then at monthly intervals for 6 months, followed by a recording at 9 months and 1 year after infarction. This study shows that PVC frequency rate in the first 3 months after MI varies widely in five subgroups of patients who can be identified using readily available clinical information. In addition, patients can be stratified into quartiles of risk for having runs of VEA during recovery from MI.

MATERIALS AND METHODS

Patients for this study were admitted between November 1971 and June 1975 to the coronary care units (CCU) of the Jewish Hospital or Barnes Hospital within 48 hours of the onset of their MI. Diagnosis of acute MI required at least two of the following three criteria: (a) a history compatible with acute MI; (b) diagnostic ECG changes consisting of the appearance of new Q waves and/or a reduction in R wave voltage; and (c) characteristic changes in serum enzyme levels [creatine kinase, SGOT, and lactic dehydrogenase (LDH) and its isoenzymes]. A variety of demographic, clinical, and laboratory parameters were obtained while the patient was in the hospital and coded on special forms (7). The information on these forms was entered into an SAS (Statistical Analysis System) computerized database (8). Extensive checks were performed to ensure consistency and accuracy of the data. When questions arose, the patient's chart was reviewed in detail by a senior cardiologist.

Analysis of the relationship of inhospital mortality to many of the following parameters has been reported previously (9). These parameters were: (a) smoking at least one pack per day of cigarettes in the last 6 months prior to acute MI; (b) diagnosis of previous diabetes mellitus, regardless of its severity; (c) history of hypertension, ascertained by the patient's knowledge of a previous diagnosis made by his physician; (d) type (nontransmural or transmural) and location (anterior, inferior, or posterior) of MI; (e) cardiac arrhythmias and IVCD during the CCU stay including PVC's at a rate of six or more per minute, APC's, atrioventricular (AV) blocks (first, second, or complete AV block), and any IVCD (right bundle branch block, left bundle branch block, left anterior or posterior hemiblocks, and nonspecific IVCD); (f) LVH by ECG; (g) cardiomegaly by chest X-ray; (h) congestive heart failure during the CCU admission, diagnosed by the presence of vascular redistribution or pulmonary congestion (mild or moderate) on chest X-ray with clinical findings of failure (rales and/or ventricular gallop), or marked pulmonary congestion or pulmonary edema with or without clinical findings or failure; and (i) cardiogenic shock, defined as a systolic blood pressure less than 90 mm Hg with evidence of peripheral vasoconstriction including sweating, cold extremities, confused sensorium, and low urinary output (less than 25 cc/hour).

Survivors of MI who were less than 71 years of age and lived within 25 miles of the medical center were eligible for the study. For the study on PVC frequency 218 patients were eligible. However, 18 patients were excluded because the site of MI (anterior, inferior, etc.) could not be determined by standard ECG criteria. Infarctions were classified as anterior (including lateral MI's) and inferior by standard ECG criteria. An additional 71 patients (for a total of 289) were analyzed for ventricular runs. Approximately 40 percent of the eligible patients were recruited for this study.

Each patient had one or more 10-hour Holter recordings (Avionics recorder, Model 350 G) obtained 2 weeks after admission (at a time when the patient was still hospitalized but ambulatory) and repeated at 1, 2, 3, 4, 5, 6, 9, and 12 months post-MI. The Argus/H computer system (10,11) was used for the detection and enumeration of all PVC's. Each computer-identified PVC was examined by a trained editor who produced annotated ECG strips of relevant sections of the tape. These strips, along with computer-generated summary information, were reviewed independently by two or more cardiologists who had no previous knowledge of the identity of the patient, the results from any other recording, or the recording session. The result of the computer analysis and editing procedure was an annotated beat-by-beat record of the long-term ECG known as an Argus/H Cycle Stream. These Cycle Streams were processed by computer programs (12) which tabulated all runs (three or more consecutive beats of ventricular ectopic origin) and identified and quantified PVC's. This information was merged with the previously mentioned parameters which described the acute phase of the patient's MI. The PVC detection rate of the Argus/H system is above 90 percent.

For statistical analysis, a logarithmic transformation of PVC rate was calculated as:

$$\log_{10} \text{PVC rate} = \log_{10} \left(\frac{\#\text{PVC per tape} + 0.5}{\text{length of tape in hours}} \right)$$

The length of the tape in hours was reduced by the amount of time during which noise made PVC detection impossible. The 0.5 added to the number of PVC's detected per tape was necessary to allow calculation of the logarithm of PVC rates of tapes with no PVC's. Although the logarithm of the PVC rate was used in the statistical analysis, for clarity of presentation, PVC rates have been converted and expressed in the more familiar units of PVC's/hour. Log transformation of PVC rates at each time period was subjected to a one-way and two-way analysis of variance utilizing SAS type IV sums of squares (13).

Parameters characterizing the patients' CCU stay were examined for their correlation with the PVC rates on the Holter recordings. Strong relationships which were clinically meaningful were further studied; three of these (site of infarct, presence of cardiomegaly, and peak level of SGOT) were used as a basis for assigning patients to one of five mutually exclusive groups.

The clinical features of patients who had at least one run during the first year after MI were compared with those of survivors who did not have this arrhythmia during the same period. Ventricular runs found on tapes made 2 weeks to 3 months following the MI were defined as "early runs," and those found 4 to 12 months post-MI were defined as "late runs." Clinical features observed during the acute phase of MI were also examined for association with the presence of either early or late runs.

We used the odds ratio ω (14) to indicate the strength of the association between clinical features and the subsequent occurrence of runs. The odds ratio is closely related to relative risk. When a clinical feature is associated with an increased risk of an event, ω is greater than 1; conversely, when the risk is decreased, ω is less than 1. Observed probability levels were computed using a standard χ^2 distribution and Fisher's exact test was used where cell expectations fell below five. A multivariate logistic equation was fit to the data to better define the relationships between clinical features of the acute MI and subsequent runs. The multivariate logistic relating clinical features to the probability of a run can be expressed as:

$$P(\text{run})=1/\ \{1+\exp(a+b1*x1+b2*x2 + . . . + bn*xn)\}$$

where P(run) is the probability of developing a run, where x1, x2, . . . xn are clinical features, and the coefficients a and b1, b2, . . . bn are estimated from the data.

RESULTS

PVC Frequency During First 3 Months Following Infarction

Of the 200 patients in whom the location of the infarct could be determined, 156 (78 percent) were male, 86 percent were white, 18.5 percent diabetic, and 46.5 percent had a history of cigarette smoking during the previous 6 months. The mean age was 56 ± 9.2 years (± 1 S.D.), and 16.5 percent had a history of one or more previous myocardial infarctions. Almost all of the patients (92.5 percent) had PVC's during their stay in the CCU and 46.5 percent had PVC rates exceeding 5/minute. One hundred and seventy-five (87.5 percent) of the infarcts were transmural. Almost half (49.5 percent) of the patients demonstrated congestive heart failure, cardiogenic shock, or pulmonary edema during the acute phase of their MI.

Figure 1 shows the cumulative distribution of PVC rates (PVC/hour) for the 568 tapes analyzed. The overall distribution very closely approaches a censored log-normal distribution, thus supporting the use of a log transform for the statistical calculations.

Ninety-seven of the 200 patients had an infarct involving one or more of the three anterior locations coded (anteroseptal, anterior, or anterolateral). Their PVC rates were less than those for patients with only inferior MI's (table 1). However, when patients with anterior infarcts were separated into those with or without radiographic evidence of cardiomegaly during the acute phase of the infarct, large differences in PVC rates were noted (figure 2 and table 1). The PVC rates of patients with anterior MI's and cardiomegaly were strikingly higher at the 2- and 3-month recording than in patients without cardiomegaly. In addition, patients with an anterior infarct and cardiomegaly had PVC rates two to three times greater at the 2- and 3-month recordings than patients with inferior infarcts. In contrast, at all time periods, patients with anterior infarcts without evidence of cardiomegaly had PVC rates less than those of patients with inferior infarcts. For patients suffering inferior infarcts, a somewhat higher PVC rate accompanied cardiomegaly during most time periods, but the differences were not statistically significant (table 1).

For each of the four groups mentioned previously (anterior with or without cardiomegaly, inferior with or without cardiomegaly), we examined the influence of the peak level of SGOT seen during the acute phase of the infarct on PVC rates. Patients within each group were subdivided into one of three subgroups according to the magnitude of peak SGOT elevation (15). Those with peak SGOT levels less than 120 IU/dl were considered as suffering "small infarcts"; those with SGOT levels between 120 and 240 units were considered as having "medium infarcts"; and those with SGOT levels greater than 240 were considered as having "large infarcts." Patients with large infarcts tended to have higher PVC rates within each of the four groups. This effect was more pronounced in patients with anterior infarctions.

The increase in PVC rates with time seen in patients with anterior infarctions and cardiomegaly was most marked in those whose infarcts were also large. Similarly, the lower PVC rates seen in patients with anterior

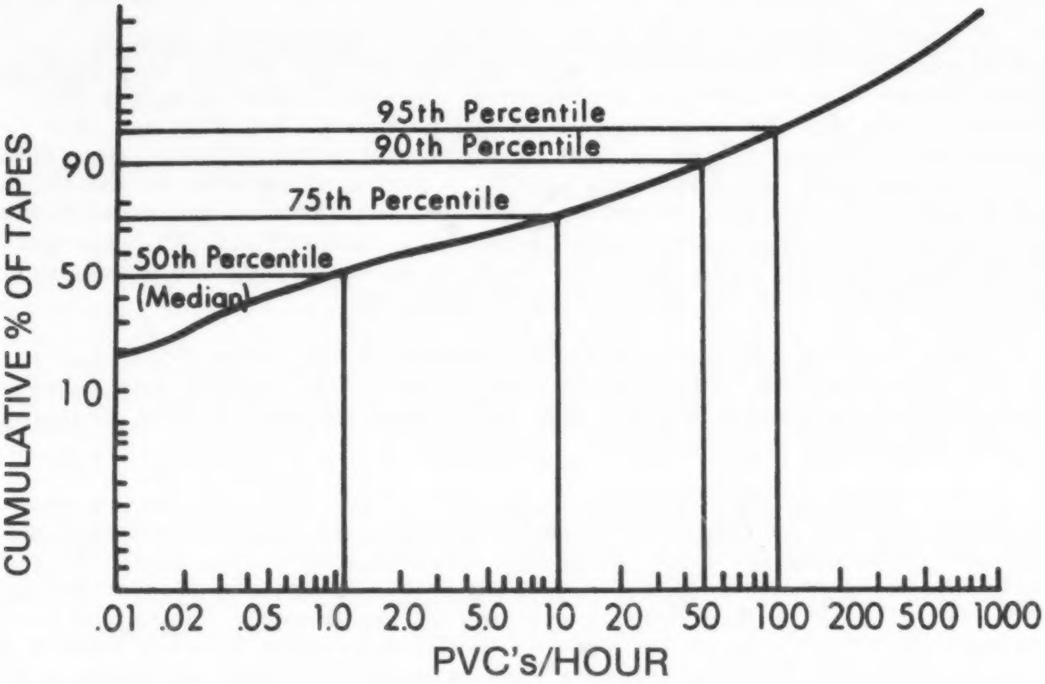

FIGURE 1. Cumulative distribution of average premature ventricular complex (PVC) rates for 568 Holter tapes obtained 2 weeks to 3 months following acute myocardial infarction plotted on a log probability scale. Note that the overall distribution is close to a straight line, indicating a log normal distribution.

infarctions but without cardiomegaly were most pronounced in those who also had "small infarcts." By combining peak SGOT with cardiomegaly and infarct location, five groups were identified (16). Group 1 consisted of 18 patients with anterior infarcts, cardiomegaly during the acute phase of their infarct, and a peak SGOT greater than 240. Group II consisted of 18 patients with anterior infarcts and cardiomegaly during the acute phase, but with peak SGOT level less than 240. Group III consisted of 103 patients with inferior but not anterior infarcts. Group IV consisted of 44 patients with anterior infarcts, no evidence of cardiomegaly, and peak SGOT levels greater than 120. Group V consisted of 17 patients with anterior infarcts, no evidence of cardiomegaly, and peak SGOT levels less than 120 units.

TABLE 1. Average Premature Ventricular Complex (PVC) Rates*

Patient groups	Recording periods				Total number of tapes	Number of patients
	2 wk	1 mo	2 mo	3 mo		
Anterior						
Cardiomegaly	0.86(32)**	1.38(21)	4.45(28)	7.68(21)	102	36
No cardiomegaly	0.28(43)	0.76(41)	0.76(46)	0.80(43)	173	61
Total anterior	0.45(75)	0.93(62)	1.49(74)	1.68(64)	275	97
Inferior						
Cardiomegaly	2.17(31)	3.83(25)	1.82(31)	2.56(25)	112	37
No cardiomegaly	1.19(47)	3.72(44)	1.55(45)	2.27(45)	181	66
Total inferior	1.51(78)	3.76(69)	1.66(76)	2.37(70)	293	103
GRAND TOTAL	153	131	150	134	568	200

* PVC's/hr, averaged over the entire tape and then averaged over all tapes in each category.

**The number of recordings analyzed is given in parentheses.

For patients with inferior infarction, further subdivision based on presence or absence of cardiomegaly or peak SGOT either alone or together did not result in subgroups with different PVC rates. Thus, all patients with inferior infarction are presented together as one group.

The average PVC rates over time for each of these five groups are shown in figure 3. Particularly striking is the greater than 300-fold difference in PVC rate between patients with anterior infarcts, cardiomegaly, and SGOT > 240 (group I), and patients with anterior infarcts but no cardiomegaly and SGOT < 120 (group V) at the 3-month recording.

Ventricular Runs During Recovery From MI

A total of 1,808 tapes were analyzed from 289 patients. The percentage of tapes with at least one run for each recording session is depicted in

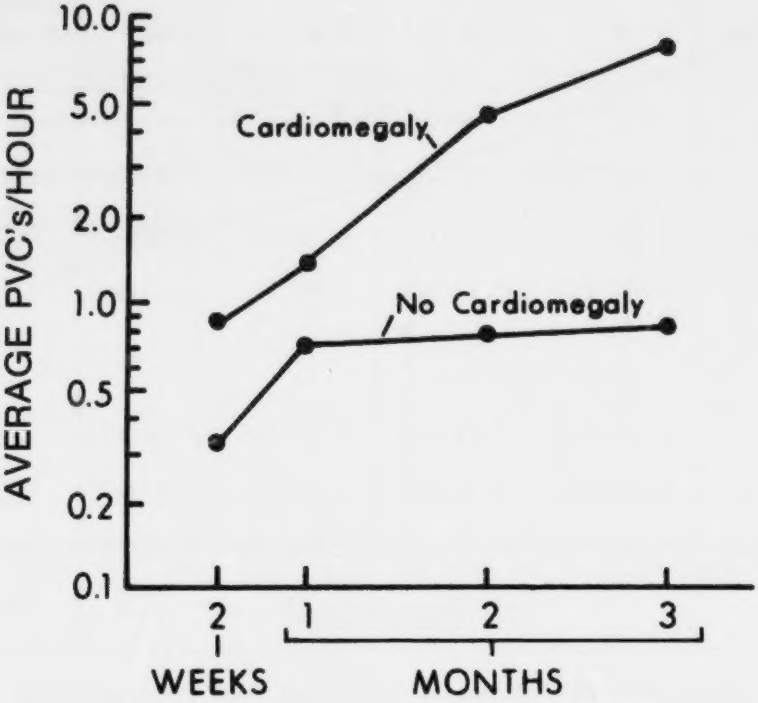

FIGURE 2. Mean premature ventricular complex (PVC) rates for patients with anterior infarcts at 2 weeks to 3 months following acute myocardial infarction. Cardiomegaly has a marked effect on PVC rates.

figure 4. The lowest frequency of runs (3.4) percent was at the 2-week recording session while the patients were still hospitalized. The PVC frequency was also lowest at the 2-week recording session. During the posthospital phase, the prevalence of runs increased to 11.5 percent at 1 month. From 2 months to 1 year, runs were found at any recording session in about 7 percent of the patients. Eighteen of the 101 tapes (18 percent) which followed a tape with a run contained one or more runs, while only 6.5 percent (83 of 1,270) of tapes following a tape without a run showed a run ($\omega = 3.10$, $p < 0.001$). Thirty-nine percent (16 of 41) of tapes following a tape with two or more runs had a run, while only 3 percent (2 of 60) of tapes following a tape with

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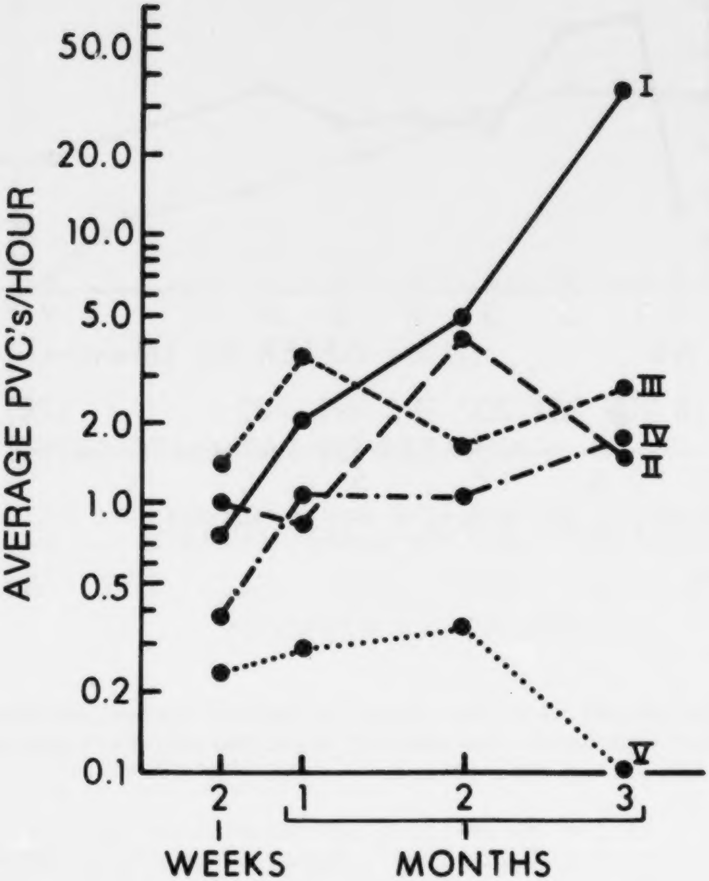

FIGURE 3. Mean premature ventricular complex (PVC) rates for five groups of patients (see text) at 2 weeks to 3 months following acute myocardial infarction (MI). While patients with anterior infarcts, cardiomegaly, and serum glutamic oxaloacetic transaminase (SGOT) > 240 had the highest PVC rates, patients with anterior MI's without cardiomegaly and SGOT < 120 had the lowest.

only one run showed one or more runs. This demonstrates that patients who have runs, particularly two or more at a given recording session, are at greater risk of having a run on the next recording.

Figure 5 shows the percentage of patients with a run on one or more tapes (excluding the 2-week session) as a function of the number of tapes analyzed for that patient. As expected, the percentage of patients with one

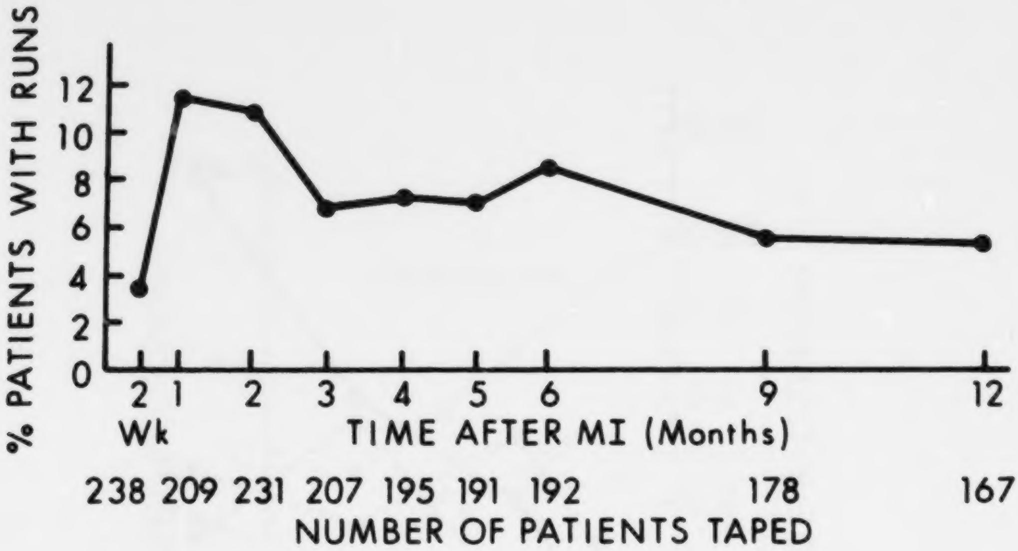

FIGURE 4. Percent of patients with runs during the year following their myocardial infarction (MI). Note the marked increase in run frequency between 2 and 4 weeks.

or more runs increased with the number of Holter recordings analyzed. Over 30 percent of the patients who had all eight outpatient tapes analyzed had a run at some time during that period.

Sixty-three of the 288 patients (22 percent) had early runs and 49 of 233 patients (21 percent) had late runs. Eighty-eight of the 289 patients (30 percent) had either early or late runs. Forty-four percent (24 of 55) of the patients with early runs (and at least one recording in the late period) had late runs as well, while only 14 percent of the patients (25 of 177) without early runs had late runs ($\omega = 4.71$, $p < 0.001$). Thus, the presence of runs 2 weeks to 3 months after MI was strongly associated with the presence of runs on tapes taken 4 to 12 months post-MI.

Demographic and clinical features which had a significant correlation with runs are shown in table 2. Coronary risk factors (smoking, hypertension, and diabetes) and previous MI were only weakly associated with runs during the postinfarction period. Neither sex, age, nor transmural MI was associated with a significant increase in risk of runs. However, indicators of pump failure (e.g., congestive heart failure, cardiomegaly, or LVH) or of electrical instability (APC's, PVC's at a rate greater than 5/minute, and AV block and IVCD) were associated with an increased prevalence of runs from 2 weeks to 1 year post-MI. The degree of association between clinical features of the MI and runs was usually greater with early runs than with late runs. Although the prevalence of posterior MI was low in this series (5 percent), patients with posterior MI appeared to have a decreased risk of runs.

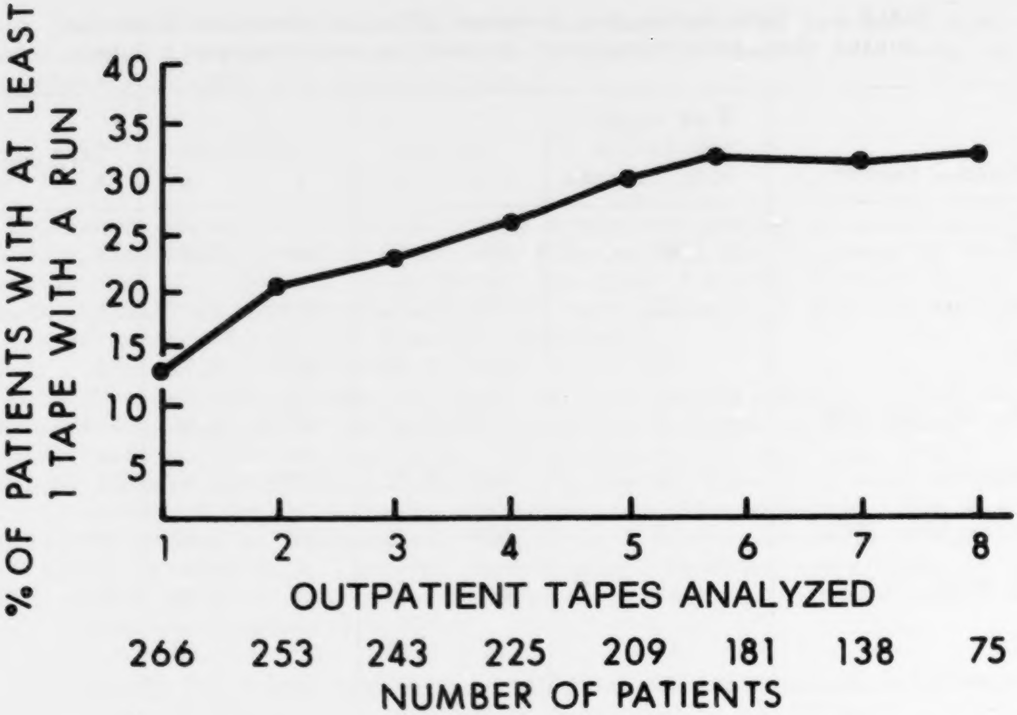

FIGURE 5. Percent of patients with at least one run. The percent of patients with runs increases with the number of recordings. However, there is little or no increase after six recordings.

The variables shown in table 2 were considered for inclusion in the multivariate logistic equation. By utilizing a special multiple regression technique (17) in which all possible combinations of variables were evaluated, we reduced the number of variables to a smaller set in which each variable contributed in a meaningful manner. We used the maximum likelihood estimates of the multivariate logistic (18) to make final refinements of the model.

A doubling of the peak LDH produced more than a doubling of the risk. The slope of this function was greater for patients with a history of previous MI. Cardiomegaly for males, LVH for patients with their initial MI, and IVCD for patients with nonanterior MI's were combinations of clinical features which independently contributed to the risk of having runs, each combination having a partial odds ratio over two.

The patients were ranked by the logistic model which predicted their probability of having runs. The 25 percent of patients at highest risk were grouped into the highest quartile numbered 1, the next 25 percent into quartile 2, etc. The average predicted risk from the model for the early as well as the late runs along with their observed prevalence for each quartile is shown in table 3. The close correspondence demonstrates the good fit of the

TABLE 2. Odds Ratios for Selected Clinical Features Observed During the Acute Myocardial Infarction and Subsequent Runs

Clinical feature	% of total population with feature	Any run %	Any run ω	Early run %	Early run ω	Late run %	Late run ω
CHF	45	36	1.60	28*	1.86	27	1.84
CM	35	38*	1.74	32**	2.33	29	1.90
LVH	14	46*	2.24	39**	2.72	32	1.99
CHF, CM, and LVH	7	57**	3.37	43*	2.96	47**	4.93
Any IVCD	20	42*	1.91	37**	2.63	23	1.17
Nonspecific IVCD	7	52*	2.73	48**	3.67	43*	3.07
Any Heart Block	11	35	1.29	29	1.54	32	1.92
APC	35	33	1.17	28*	1.70	21	0.99

* $p < 0.05$. ** $p < 0.01$.

CHF = Congestive heart failure. CM = Cardiomegaly. APC = Atrial premature complex. LVH = Left ventricular hypertrophy. IVCD = Intraventricular conduction defect.

TABLE 3. Observed Percent Risk of Early Runs, Grouped by Quartile of Risk From Logistic Solution

Quartile	Average predicted risk of early run (range)	Observed prevalence of early run	Observed prevalence of late run
1	49.0 (29.5-90.4)	49.3	29.6
2	21.8 (16.5-29.1)	20.8	22.6
3	12.5 (8.7-16.2)	13.7	18.6
4	5.0 (0.1-8.6)	4.2	13.8

model. Individuals at high risk for early runs also were at increased risk for late runs. Although the model was not intended to predict late runs, it nevertheless predicted them reasonably well.

DISCUSSION

This study shows that clinical features recorded during the acute phase of an MI are excellent predictors of both PVC rate and the incidence of runs in survivors of MI. The PVC rates during the first 3 months postinfarction and runs not only in this period but in the late period 4-12 months postinfarction can be predicted from the clinical features.

Both PVC frequency and the incidence of runs increase markedly from the 2-week recording made while the patient is in the hospital to subsequent recordings made outside the hospital. The prevalence of runs went from 3 percent at 2 weeks post-MI to 11 percent at 1 month. Runs were seen in approximately 7 percent of patients during subsequent recording sessions. The average PVC frequency tended to increase over the first 3 months particularly for patients with cardiomegaly. Several investigators studying arrhythmias in the recovery period after MI have also noted an increase in arrhythmias once the patient leaves the hospital (19,20).

The highest PVC rates were seen in patients with anterior MI, cardiomegaly, and SGOT > 240. Patients with anterior MI's and SGOT < 120 without cardiomegaly had the lowest PVC rates of the five groups. All patients with inferior MI's had intermediate PVC rates during the first 3 months. It is of note that cardiomegaly and enzyme level had relatively little effect on PVC frequency in patients with inferior infarction. One possible explanation for this finding is that some enzyme release in patients with inferior infarction is due to right ventricular necrosis. Death of this portion of myocardium may be less arrhythmogenic than left ventricular infarction.

Like PVC rates, the incidence of runs was related to clinical features recorded during the acute infarction. Twenty-two percent of patients had runs during the early convalescent period and 21 percent during the late. With repeated monitoring, nearly a third of the patients exhibited a run sometime during the first year postinfarction. This incidence is higher than others have reported (2,3,21) and is probably due to more frequent recording sessions on each patient in our study (average 6.3 tapes/patient).

We found, as have others (2,4), that the prevalence of runs is increased in patients with significant ventricular dysfunction. Patients who during their acute MI had either congestive heart failure, cardiomegaly, greater enzyme elevation, ECG evidence of LVH, IVCD, APC's, or PVC's > 5/minute had an increased risk of runs in the posthospital period. Age, sex, hypertension, diabetes, and a history of smoking had little influence on the incidence of runs.

As in PVC frequency rates, the degree of enzyme elevation had a strong influence on the risk of runs in the posthospital period. Furthermore, multivariate logistic analysis showed that peak LDH or peak SGOT was an important independent predictor of runs. Using the logistic model, we were able to group patients into quartiles of risk of runs in the early period, ranging from 3 percent to 49 percent. Although clinical features recorded during the acute infarction were somewhat less successful in identifying who would develop late runs (4-12 months postinfarction), it must be emphasized that the logistic equation was developed for the prediction of early runs. In view of this, it is gratifying that we were also able to divide patients into quartiles of risk for late runs.

CONCLUSION

Our data show a marked variability in PVC frequency rate and a high prevalence of runs in survivors of acute infarction. The prevalence of runs and the PVC rate are related to clinical features recorded during acute infarction. Patients with anterior infarctions with high peak enzyme levels and cardiomegaly have the highest PVC frequency rates after discharge from the hospital while those with anterior infarcts without cardiomegaly and with peak SGOT $<$ 120 have the lowest. Runs in both the early and late convalescent periods are related to a number of clinical features including peak enzyme levels, cardiomegaly, congestive heart failure, LVH, and certain arrhythmias recorded during acute infarction. By employing the technique of multivariate logistic analysis, we were able to group patients into quartiles with markedly varying incidences of runs. In addition, by using readily available information which characterized the patients' acute MI, we were able to identify prospectively patient groups with widely disparate PVC frequencies. The ability to identify readily such subgroups of patients may be of value not only in evaluating antiarrhythmic interventions but also in establishing the independent effect of arrhythmias on survival postinfarct.

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RESULTS OF A STUDY ON THE PREVENTION OF SUDDEN DEATH IN ISCHEMIC HEART DISEASE PATIENTS BY MEANS OF PROPRANOLOL (PRELIMINARY DATA)

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ABSTRACT. In order to develop approaches to the prevention of sudden cardiac death, 506 men born 1919-1942 and all suffering from ischemic heart disease (IHD), were examined. Two-stage screening, including 24-hour electrocardiographic (ECG) monitoring and a treadmill test, was used to select 98 men with grade 2 or higher ventricular extrasystoles and without countraindications for long-term treatment with obsidan (an analog of propranolol). The experimental group (treated with obsidan) consisted of 46 men, and the control group (which received no active treatment) consisted of 52 men. The groups were randomized according to age, nature of myocardial infarction, presence of angina, and primary IHD risk factors. Obsidan treatment began with 40 mg/day, and the dosage was gradually increased. The daily dose was considered adequate when heart rate was stably reduced by 15-20 percent. As a result of treatment, heart rate was reduced in the experimental group by an average of 19.2 percent; the reduction in the control group was 1.7 percent. Systolic and diastolic pressures dropped in the experimental group by 11 percent, and increased in the control group by 0.6 and 1.9 percent, respectively. Treatment with obsidan resulted in some improvement in the course of the angina, manifested by an increase (by 16 percent) in the number of patients without angina of effort or at rest. This figure increased by only 6 percent in the control group. Deterioration in angina dynamics was observed in 4 percent and 21 percent of the cases in the experimental and control groups, respectively. When the experimental and control groups were randomized according to initial nature of rhythm disorder, a tendency was observed towards a reduced number of patients with grades 4-5 in the experimental group.

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INTRODUCTION

Our study, performed within the framework of the US-USSR cooperation in Problem Area 5, Sudden Death, has shown that patients with documented chronic IHD (following myocardial infarction) and with complex forms of ventricular extrasystoles (polytopic, couplets, salvoes, and early extrasystole), found by 24-hour monitoring, have a comparatively high risk of sudden death. Patients for whom these types of rhythm disorders are not recorded have a low risk of sudden death (1). Presence of angina of effort in these patients also indicates a poorer prognosis. Therefore, the use of preparations which have simultaneous antiarrhythmic and antianginal effects should theoretically be promising from the standpoint of preventing sudden death. β-Adrenoreceptor blockers have these properties.

Two significant studies have been conducted in which these preparations were used for long-term treatment of IHD patients. One study in which the selective β-blocker practolol was used was stopped due to the development of severe complications (2). In another study, patients who had suffered a myocardial infarction were treated with alprenolol after persons with a high risk of death were eliminated (3). The results from the first study are difficult to explain and differ from the results of the second study. In particular, why was a positive effect seen only in patients who had suffered anterior myocardial infarction? Was the result truly related to the location of the infarct, or was there perhaps a correlation with other factors not considered in the study? Were the positive results related to the antianginal or to the antiarrhythmic effect of the β-adrenoreceptor blockers? All of these questions remain unanswered.

We know that none of the available antiarrhythmic preparations is effective for all patients with rhythm disorders. It is therefore expedient to select antiarrhythmic preparations for the treatment of these patients individually. However, as we have shown earlier (4), complex grades of ventricular extrasystoles are seen with second recordings in only 14-16 percent of IHD patients. The great variability in manifestation of extrasystoles has been clearly demonstrated by Winkle (5). And, in order to determine the presence or lack of an effect of a preparation, some authors recommend continuous monitoring for a period of 2 weeks. It is unrealistic, therefore, to attempt individual selection of antiarrhythmic preparations for large-scale investigations or for extensive therapy among patients with IHD. But, such treatment is possible in a group of severely ill patients in whom attacks of ventricular tachycardia and ventricular fibrillation occur frequently.

Therefore, without abandoning the principle of individual selection of antiarrhythmic preparations for some patients, we set the primary purpose of our investigation as follows: to study the possibility of long-term treatment with propranolol of patients at high risk of sudden death, and to determine its effect on the complex grades of ventricular extrasystoles.

MATERIALS AND METHODS

Men born between 1919 and 1942 (725 subjects), who were receiving treatment in a number of hospitals in Moscow following myocardial infarction or chronic IHD, were invited to come to the Institute of Cardiology for examination. Seventy percent responded to the invitation and, after the first examination, 35 percent of those who had passed the first screening were invited to a second examination. After the second examination, only 20 percent of the total number who responded to the initial invitation were selected for long-term observation and treatment. The reasons for not including the other patients are listed in table 1.

The number of patients who were excluded because of the absence of complex grades of ventricular extrasystoles represented 32 percent of all the patients monitored.

Examination of those invited to the first screening included standard questioning to reveal angina of effort (Rose questionnaire), smoking habits, and intermittent claudication; a general examination of the patient; measurement of blood pressure twice while seated; ECG at rest and 24-hour ECG monitoring; physical exercise test on a treadmill; and determination of fasting blood glucose and blood glucose 1 hour after a 50 g glucose load.

At the second screening, the patients were questioned following an expanded questionnaire which included the Rose questionnaire as well as questions about past diseases. A general examination was then conducted with measurement of blood pressure and ECG at rest; anthropometric examination; radiographic determination of heart dimensions; determination of cholesterol, α-cholesterol, triglycerides, and glucose in the blood, fasting and 1 hour after a 50 g glucose load per os, blood creatinine, and transaminase; and clinical analysis of the blood and urine.

Thus, the study included patients with chronic IHD and complex grades of ventricular extrasystoles (grade 2 and higher according to Lown) and without contraindications for long-term treatment with propranolol. Ischemic heart disease was established on the basis of the following criteria:

- Typical angina of effort
- Documented myocardial infarction based on the criteria in the "Myocardial Infarction Register," acute period
- Presence on the ECG of data indicating myocardial infarction (1-1 and 1-2 on the Minnesota code)
- Presence of IHD based on ECG indications (4-1.2 and 5-1.2 without 3-1 on the Minnesota code).

After the second screening, the patients included in the study were randomly divided into two groups: the experimental (or treated) group, and the control (or comparison) group. The groups were randomized according to age (by decades), definite or possible myocardial infarction, presence of angina

TABLE 1. Reasons for Not Including Patients in the Long-Term Study Following the First and Second Screenings

Reason for exclusion	No. of patients (%)	
	1st screening	2nd screening
1. No Ischemic Heart Disease	11	-
2. Intermittent claudication	8	-
3. Bradycardia (less than 55/min)	6	16
4. Heart failure	9	8
5. Stroke in patient's history	7	-
6. Pulmonary disease	4	4
7. Gastrointestinal disease	10	12
8. Allergy in patient's history	4	-
9. Diabetes	2	4
10. Bundle-branch block	5	8
11. Atrial flutter	2	4
12. Atrioventricular block	1	-
13. Other severe disease	11	12
14. Other causes	10	16
15. Patient failed to make contact	10	16
TOTAL	100.0	100.0

of effort, and at least one of the primary IHD risk factors (hypertension, hypercholesterolemia, excessive smoking, low tolerance for carbohydrates, and obesity).

The patients in the treated group visited a physician in our Institute regularly (at least once every 2-3 weeks). Patients in the control group were invited to the Institute for examination only once every 6 months, and they were treated by polyclinic doctors (participating physicians).

Treatment with obsidan (an analog of propranolol) began with 10 mg given four times per day, and then the dose was gradually increased. The mean dose was 92 mg, and dosages ranged from 40 mg to 240 mg per day. The daily dose was considered effective if it produced a stable decrease in heart rate by 15-20 percent. After this bradycardiac effect was achieved, the patients were reexamined, including 24-hour monitoring.

Patients from the control group were reexamined after 6 months.

RESULTS

There are presently 98 persons under observation (46 in the experimental group and 52 in the control group). The comparative characteristics of these groups are presented in table 2.

More than half of the patients (54-58 percent) in both groups had angina of effort, and most (71-81 percent) had experienced definite myocardial infarctions. Only 4 percent had not experienced an infarction, but these were included in the study because they had typical angina of effort.

Patients included in the study manifested ventricular extrasystoles of grade 2 or higher in the initial 24-hour ECG. However, with more detailed analysis, certain differences were found between the experimental and control groups (table 3).

TABLE 2. Initial Characteristics of Patients In the Experimental and Control Groups

Group	No. of patients	Mean age (yr)	No. of patients (%)						
			Angina pectoris		Myocardial infarction			IHD risk factor	
			Yes	No	Definite	Possible	None	Yes	No
Experimental	46	49.3	54	46	81	15	4	76	24
Control	52	48.7	58	42	71	25	4	77	23
TOTAL	98	49	56	44	75	20	4	77	23

IHD = Ischemic heart disease.

TABLE 3. Comparison of the Frequency of Ventricular Extrasystoles (VE) in the Experimental Group and the Control Group, Based on Maximum Grade

VE grade	Frequency of VE (%)	
	Experimental group	Control group
2	0	2
3	58	61
4a	13	19
4b	20	6
5	9	12

Slightly more patients in the experimental group had bursts of extrasystoles, while slightly more in the control group had couplets and early ventricular extrasystoles.

Changes in heart rate were evaluated by ECG recordings at rest. Initially, the mean heart rate in the experimental group was 73 beats/min, and 70 beats/min in the control group. Obsidan reduced the frequency in the experimental group by 14.0 ± 2.0 beats/min (an average of 19.2 percent); the control group also showed a decrease in heart rate, but only by 1.0 ± 1 (1.7 percent) (figure 1). The difference between the experimental and control groups was statistically significant ($p < 0.001$). A 240 mg dose of obsidan, which was the maximum daily dose used in this study, did not achieve the desired level of decrease in heart rate in two of our patients.

During treatment, which continued with the same dose of obsidan, some decrease in the bradycardiac effect was observed (figure 2).

The effect of taking β-blockers was also documented by a decrease in the levels of systolic and diastolic pressures. The mean systolic blood pressure in the experimental group was 144 mm Hg, and the mean diastolic pressure was 92 mm Hg; in the control group, the means were 138 mm Hg and 87 mm Hg, respectively. Reexamination after treatment showed a decrease to 128 mm Hg and 89 mm Hg for systolic and diastolic pressure, respectively. In the control group, blood pressure increased slightly, to an average of 139 mm Hg and 102 mm Hg for systolic and diastolic pressure, respectively (figure 3). The increase in the mean figures, particularly in diastolic pressure, resulted mostly from a decrease among the IHD patients in the number of persons with normal blood pressure and an increase in the number of patients with hypertension (table 4).

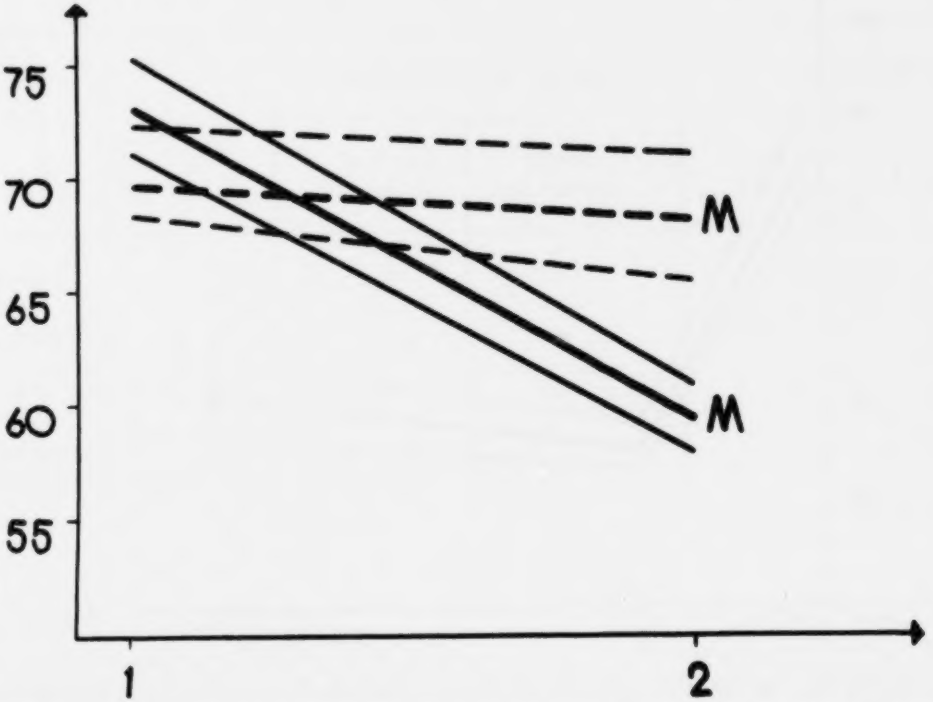

FIGURE 1. Dynamics of heart rate in the experimental and control groups. Abscissa: Examination periods. 1 = Before treatment and observation. 2 = Reexamination. Ordinate: Heart rate (beats/minute). ——— = Experimental group. - - - = Control group. M = Mean value between maximum and minimum heart rate based on ECG at rest.

Treatment with obsidan resulted in some improvement in the course of the angina, manifested as an increase (by 16 percent) in the number of patients without angina of effort or angina at rest. In the control group, this figure also increased by 6 percent.

Deterioration (i.e., appearance of new cases of angina of effort or angina at rest) was noted in the treated group in only 4 percent of the cases, as opposed to 21 percent in the control group (figure 4).

As we have noted, the groups differed somewhat initially in terms of the frequency of maximum grades of ventricular extrasystoles. Therefore, in evaluating the antiarrhythmic effect, we conducted additional randomization based on the nature of the initial arrhythmia. In the experimental group receiving treatment, we found a smaller number of patients with grades 4 and 5 ventricular extrasystoles than in the control group (6.7 percent and 20 percent, respectively). However, approximately the same number of patients in each group had grades 0 or 1.

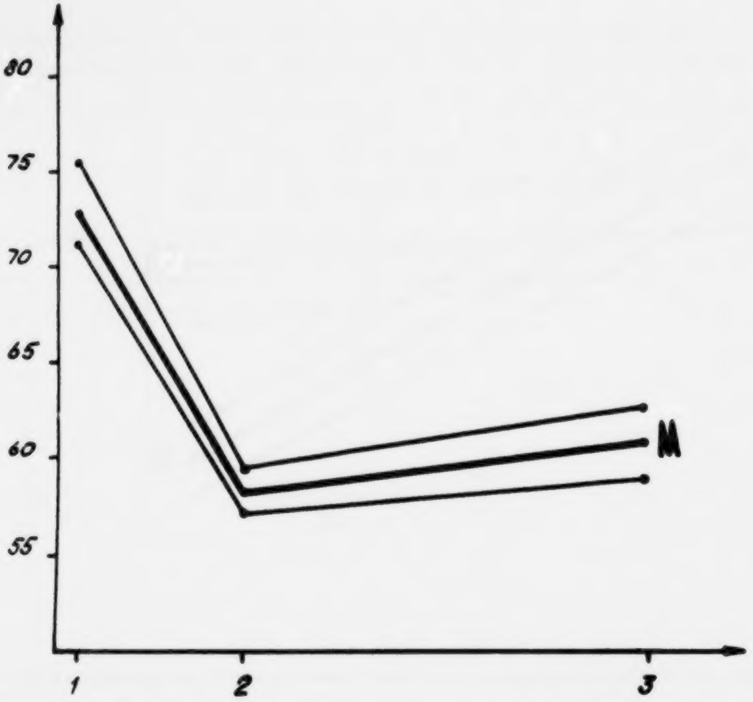

FIGURE 2. Dynamics of heart rate in patients during 6 months of treatment. Abscissa: Examination periods. 1 = Before treatment. 2 = Upon completion of selection of individual dose, after 3-6 months of treatment. Ordinate: Heart rate (beats/minute). M = Mean value.

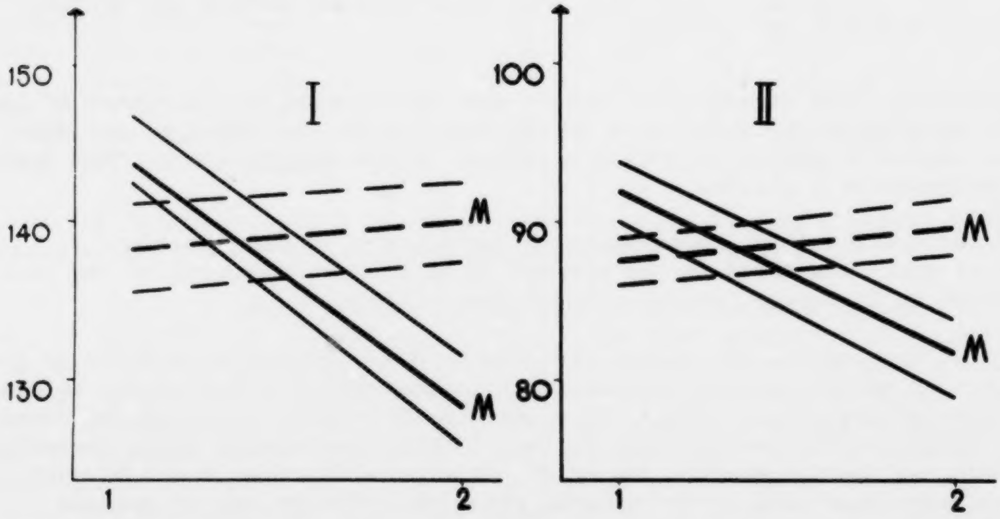

FIGURE 3. Dynamics of systolic (I) and diastolic (II) pressures in the experimental and control groups. Abscissa: Examination periods. 1 = Before treatment and observation. 2 = Reexamination. Ordinate: Blood pressure (mm Hg). See figure 1 for other designations.

TABLE 4. Dynamics of Frequency of Arterial Hypertension (in %)

Blood pressure	Experimental group		Control group	
	Initial reading	After treatment	Initial reading	After 6 months
Normal	25	62	56	41
Borderline (≥ 140/90 mm Hg and < 160/95 mm Hg)	33	25	22	22
Elevated (≥ 160/95 mm Hg)	42	13	22	37

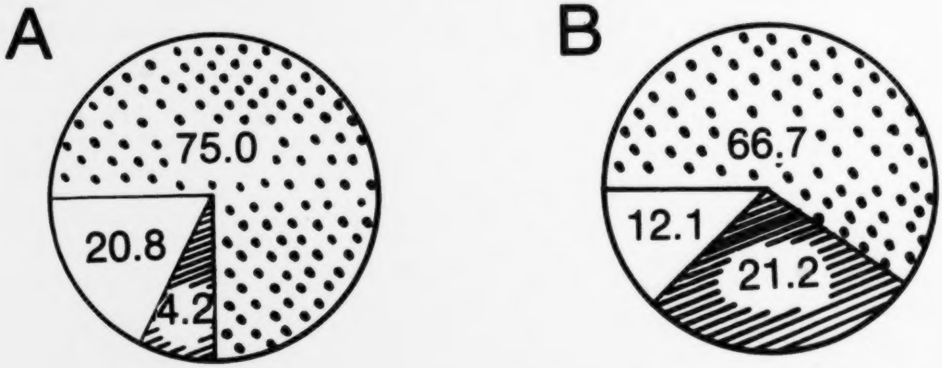

FIGURE 4. Dynamics of angina of effort in chronic IHD patients treated with obsidan (A) and in the control group (B). □ = Improvement. ▨ = Deterioration. ░ = No change.

CONCLUSIONS

The data obtained in our study are considered preliminary. After examination and long-term treatment with a nonselective β-adrenoreceptor blocker, complex grades of ventricular extrasystoles were observed by one-time 24-hour ECG monitoring in about 20 percent of the chronic IHD patients. Daily dosage of 40-240 mg obsidan led to a decrease (by an average of 19 percent) in the heart rate of most patients as compared to their initial heart rate.

Treatment with obsidan of patients with IHD and arterial hypertension led to a drop in blood pressure to normal values in approximately one-half of the cases. Treatment with obsidan (for an average of 2 months) in persons who had experienced myocardial infarction led to a decrease in the number of patients with new cases of angina of effort or angina at rest.

The antiarrhythmic effect of obsidan is not as pronounced in chronic IHD patients, and a larger number of patients should be observed to determine whether or not obsidan has such an effect.

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PART II

CARDIAC ELECTROPHYSIOLOGY

DIFFERENT ELECTROPHYSIOLOGICAL RESPONSES OF CANINE ENDOCARDIUM AND EPICARDIUM TO COMBINED HYPERKALEMIA, HYPOXIA, AND ACIDOSIS

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ABSTRACT. Why ventricular endocardium (EN) develops less conduction delay than epicardium (EP) during acute myocardial ischemia (MI) following coronary artery occlusion is not clear. To determine if EN and EP have intrinsically different sensitivities to metabolic alterations that occur during MI ($\downarrow$ pO_2, $\downarrow$ pH, $\uparrow$ $[K^+]_o$), mongrel dogs (n = 35) were anesthetized with secobarbital and a 1.5 x 1.5 cm^2 of right ventricle was excised. A cut through the midwall divided the EN from the EP. Both pieces, now separated, were pinned to the floor of the same chamber with the EN and EP surfaces up, superfused with Tyrode's solution ($pO_2 > 400$, pH 7.37, $[K^+]_o$ 4.0) or altered Tyrode's (AT) ($pO_2 < 50$, pH 6.85, $[K^+]_o$ 8.0) at 37° C and stimulated at 2 Hz. Action potentials from EP muscle and EN muscle or Purkinje fibers were recorded simultaneously using standard microelectrode techniques. During AT superfusion, all cells showed progressive decreases in action potential duration, amplitude, dV/dt, and resting potential and increases in activation time. The magnitude of these changes were EP muscle > EN muscle > Purkinje fibers. Refractoriness outlasting repolarization was of greater magnitude in EP than in EN. Of the 18 EP muscle cells, 15 were unresponsive after 15 minutes of AT, but 14 of 16 EN muscle cells and 13 of 13 Purkinje cells were still responsive. These data indicate that EP is more severely affected by $\downarrow$ pO_2, $\downarrow$ pH, and $\uparrow$ $[K^+]_o$ than EN in vitro and may partially explain differential effects observed during MI in vivo.

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INTRODUCTION

For unknown reasons, conduction in the left ventricular subendocardium remains relatively preserved during acute myocardial ischemia in vivo, when conduction in the subepicardium has undergone progressive delay and fractionation (1-5). Since nonuniform transmural conduction delay during acute myocardial ischemia could predispose to the development of lethal reentrant ventricular arrhythmias, the cellular electrophysiological basis for such delay might have important therapeutic implications. The purpose of the present study was to investigate whether the electrical activity of epicardial muscle, endocardial muscle, and Purkinje fibers is affected differently by metabolic alterations known to occur during acute coronary artery occlusion. The alterations tested were elevated extracellular potassium and reduced oxygen tension and extracellular pH (6-7).

METHODS

General

Mongrel adult dogs weighing 15-25 kg were anesthetized with sodium secobarbital (30 mg/kg intravenously), and their hearts were rapidly excised and placed in cool Tyrode's solution. A 1.5 x 1.5 cm^2 of myocardium from the base of the right ventricle was dissected free and split into two halves by an incision through the midmyocardium. The two pieces, now separated, were pinned to the wax bottom of a plexiglass superfusion chamber with the epicardial and endocardial surfaces facing upwards. In some experiments, single right ventricular endocardial preparations containing free-running false tendons or single right ventricular papillary muscle preparations were used.

The preparations were superfused at 37 ± 0.5° C, pH 7.37 ± 0.05, with oxygenated (95 percent O_2, 5 percent CO_2) Tyrode's solution, composed (in millimoles) of: $MgCl_2$, 0.5; NaH_2PO_4, 0.9; $CaCl_4$, 2.7; NaCl, 137.0; $NaHCO_3$, 12.0; KCl, 4.0; and glucose, 5.0. The oxygen tension was 400-600 mm Hg.

Each piece of myocardium was stimulated independently at a cycle length of 500 msec with bipolar Teflon-coated stainless steel electrodes. Stimuli were rectangular pulses of 2 msec duration delivered at an intensity of one and one-half times the diastolic threshold and were obtained from a pulse generator through an isolation transformer (Digipulser model 830, Isopulser model 850, W-P Instruments, Inc.)

Transmembrane and bipolar extracellular potentials were recorded using standard techniques. The recordings were displayed on a memory oscilloscope (Tektronix D15), photographed with a Polaroid camera (C-59), and recorded on magnetic tape (Hewlett-Packard model 3960).

Measurements

The action potential characteristics measured were resting membrane potential (RMP), action potential amplitude (APA), action potential duration at 50 and 90 percent of repolarization (APD_{50} and APD_{90}), the maximum rate of rise of phase zero (dV/dt), activation time (ACT), and the effective refractory period (ERP).

Activation times were determined by the time interval between the upstroke of the stimulus artifact and the maximum dV/dt deflection, except in false tendons during superfusion with altered Tyrode's solution when they were measured between two microelectrodes placed at opposite ends of a Purkinje fiber. The ERP was determined using the extrastimulus technique (8).

Membrane responsiveness in endocardial, epicardial, and papillary muscle was estimated by plotting dV/dt as a function of the take-off potential (TOP) at various points in phase three and four of the action potential during premature stimulation. Membrane responsiveness was also estimated by plotting the relationship between RMP and dV/dt during control periods and at various times during superfusion with altered Tyrode's solution, when spontaneous changes in RMP occurred as the result of superfusion.

Protocols

Following 45-60 minutes of equilibration in normal Tyrode's solution the preparations were superfused with altered Tyrode's solution maintained at 37° C. Altered Tyrode's solution had the same millimolar composition as normal Tyrode's solution, with the exception of KCl - 8.0 mM, $NaHCO_3$ - 5.45 mM, and NaCl - 150.0 mM. Altered Tyrode's solution was bubbled with 95 percent N_2, 5 percent CO_2, resulting in a pH of 6.85 ± 0.05 and a $pO_2 < 50$ mm Hg. The changes in KCl, oxygen, and hydrogen ion content in altered Tyrode's solution are similar to those reported to occur during acute myocardial ischemia (6,9). Altered Tyrode's solution was superfused for 15 minutes, followed by 60 minutes of washout with normal Tyrode's solution. In 13 preparations, altered Tyrode's solution was superfused for 60 minutes to determine the electrophysiological consequences of prolonged exposure.

Statistical analysis was performed using Student's *t*-test for paired or unpaired data, where appropriate (10).

RESULTS

Effects of Altered Tyrode's Solution on Transmembrane Potential Characteristics

Figure 1 illustrates the effects of superfusion with altered Tyrode's solution (AT) on action potentials recorded from endocardial (EN - top recording) and epicardial (EP - bottom recording) muscle cells. During control, the duration of the action potential recorded from the epicardium was shorter than the duration of the action potential recorded from the endocardium and displayed a prominent notch between phases one and two. Superfusion with altered Tyrode's solution progressively shortened APD, reduced RMP, APA, and dV/dt, and prolonged ACT in both tissues. The separation between phase zero and two of the epicardial cells after 13 minutes of superfusion with altered Tyrode's solution was a characteristic response of epicardial and papillary muscle, but was not seen in

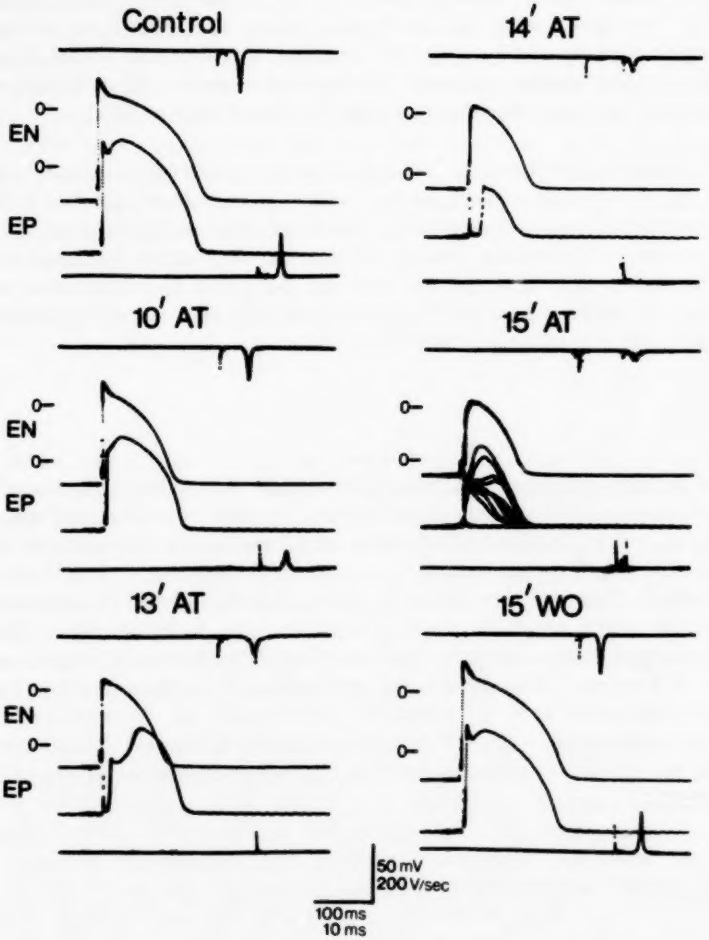

FIGURE 1. Effects of altered Tyrode's solution (AT) and washout (WO) on the action potential characteristics of canine endocardial (EN) and epicardial (EP) muscle cells. The top and bottom tracings are the dV/dt recordings for EN and EP, respectively. Superfusion with AT resulted in progressive loss of resting membrane potential, action potential amplitude, action potential duration, and dV/dt, with increasing activation time. After 15 minutes of superfusion with AT (15' AT), EP was unresponsive to stimulation at the original stimulus intensity and duration. Increasing stimulus duration from 2 to 12 msec elicited action potentials of diminishing amplitude. Both cells returned toward control following 15' WO. Top and bottom calibrations correspond to action potential and dV/dt recordings, respectively.

endocardial cells. The plateau of the epicardial cell was suppressed after continued superfusion of altered Tyrode's solution, and only a smaller action potential remained (14' AT). At the time when the epicardial cell became unresponsive (15' AT), the stimulus duration was increased from 2 msec to 12 msec. This elicited an action potential with a more prominent plateau that rapidly diminished in amplitude. Further increases in stimulus duration produced similar patterns, but the initial action potential was reduced in amplitude and the decrease in amplitude occurred more rapidly. Finally, complete inexcitability of the epicardial cell ensued regardless of stimulus intensity, at a time when the endocardium still responded to a stimulus of 2 msec duration.

The effects of altered Tyrode's solution were reversed following 15 minutes of washout. Although RMP, APA, dV/dt, and ACT returned to control values, APD_{50} and APD_{90} were prolonged following 20 minutes of washout in both tissues.

Changes in cellular action potential characteristics during superfusion with altered Tyrode's solution appeared to correlate with bipolar electrogram alterations (figure 2). Activation delay, loss of amplitude, and fractionation of electrograms were more severe in epicardium than in endocardium.

In three preparations, the same endocardial and epicardial impalements were maintained throughout two 15-minute periods of superfusion with altered Tyrode's solution, separated by 30 minutes of washout. The RMP and APA were slightly increased in both cell types during washout following initial superfusion with altered Tyrode's solution, and the electrical changes induced by the second superfusion with altered Tyrode's solution were less marked, particularly in the endocardial cells.

The effects of acute and prolonged exposure of Purkinje cells to altered Tyrode's solution are illustrated in figure 3. Marked depression of RMP, APA, APD, and dV/dt occurred after 15 minutes of superfusion with altered Tyrode's solution. These changes partially reversed over the next 45 minutes of continued superfusion with altered Tyrode's solution. Stabilization or partial reversal of the effects of altered Tyrode's solution were seen in three additional experiments in which 60-90 minute impalements of the same cell were obtained. In addition, improvement of Purkinje fiber action potentials occurred in two of two preparations superfused with altered Tyrode's solution which did not contain glucose. In a similar fashion, endocardial muscle cell action potentials partially recovered in three of four preparations during 20-60 minutes of superfusion with altered Tyrode's solution containing glucose. Epicardial cells (n = 5) and papillary muscle cells (n = 4) showed only slight or no improvement with prolonged superfusion. Following 10 minutes of washout, the configuration of each type of action potential returned toward control contours, although APD was increased.

The average values (± SEM) for RMP and APA during control, at 5-minute intervals during superfusion with altered Tyrode's solution, and at various times during washout for endocardial, epicardial, and Purkinje fibers are given in figure 4. A similar format is used to display changes in APD_{50} and APD_{90} (figure 5) and dV/dt and ACT (figure 6). These data are taken from 18 epicardial preparations, 18 corresponding endocardial preparations (9 in which endocardial muscle electrical activity was recorded and 9 in which Purkinje fiber electrical

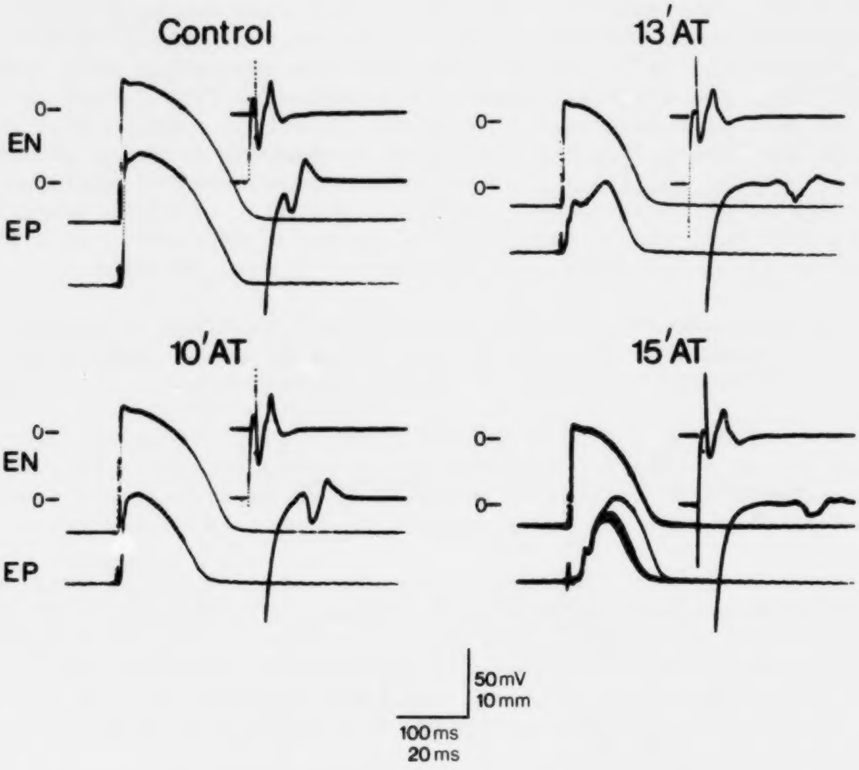

FIGURE 2. Effects of altered Tyrode's solution (AT) on the action potential (left) and bipolar electrogram (right) characteristics of endocardial (EN) and epicardial (EP) muscle cells. Action potential changes were similar to those depicted in figure 1 and the severity of the action potential changes correlated with the degree of delayed activation, loss of amplitude, and fractionation of the bipolar electrograms. The top and bottom calibrations correspond to action potential and electrogram recordings, respectively.

activity was recorded), and 5 right ventricular endocardial preparations containing free-running false tendons from which endocardial muscle (seven cells) and Purkinje fiber (four cells) action potentials were recorded. Data from the latter preparations did not differ significantly from data obtained in the other endocardial and Purkinje preparations, and are therefore combined. The raw data for nine papillary muscle preparations (16 cells) are given in table 1.

After 5 minutes of superfusion with altered Tyrode's solution, 1 of 18 epicardial cells was unresponsive; after 10 minutes, 9 of 18 epicardial cells and 5 of 16 papillary muscle cells were unresponsive; and, after 15 minutes, 15 of 18 epicardial cells and 11 of 16 papillary muscle cells were unresponsive. These cells were not included in the data analysis other than for measurement of RMP. All 13 Purkinje cells were responsive throughout the 15-minute superfusion with altered Tyrode's solution, while 2 of 16 endocardial muscle cells were unresponsive at 15 minutes. All cells were excitable after 5 minutes of washout.

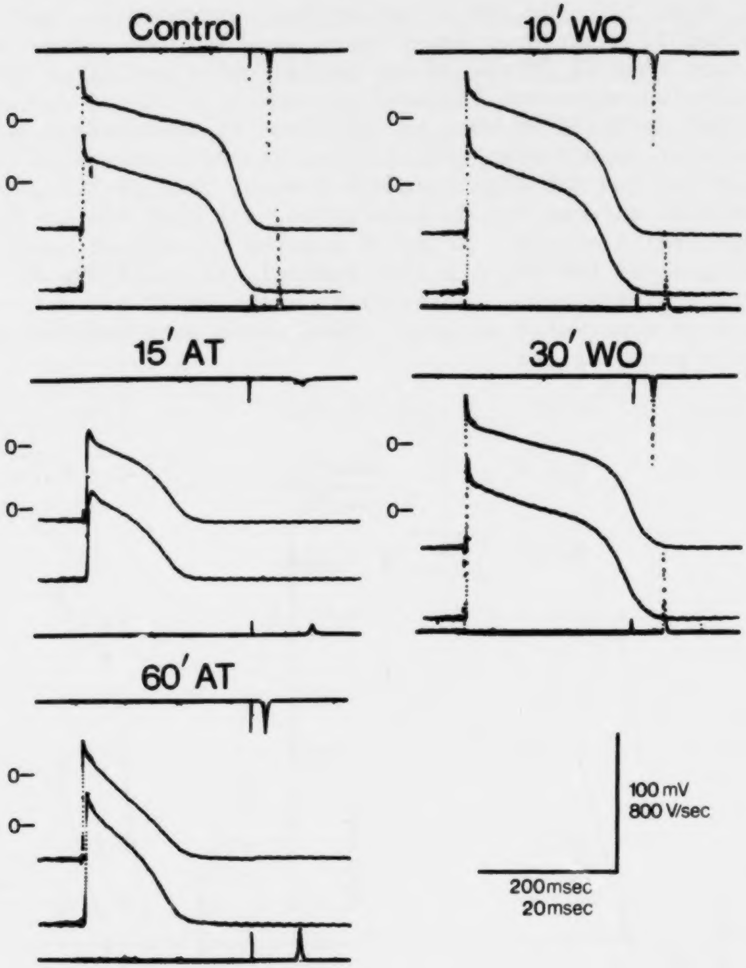

FIGURE 3. Effects of altered Tyrode's solution (AT) and washout (WO) on the action potential characteristics of canine Purkinje fibers. After 15 minutes of superfusion with AT (15' AT), resting membrane potential, action potential amplitude, action potential duration, and dV/dt were reduced. Activation time was prolonged, but interelectrode conduction time was only slightly longer than control. Between 20 and 60 minutes of superfusion with AT there was a partial return of action potential characteristics toward control (60' AT). Restoration of action potential configurations occurred following WO, although action potential duration was prolonged and dV/dt was diminished relative to control (30' WO). The top and bottom calibrations correspond to action potential and dV/dt recordings, respectively.

During superfusion with altered Tyrode's solution, the four types of cells showed progressive loss of RMP and APA that returned toward control with washout (figure 4 and table 1). The RMP of epicardial, endocardial, and papillary muscle was similar after 15 minutes of superfusion with altered Tyrode's solution, in spite of the fact that 15 of the 18 epicardial cells and 11 of the 16 papillary muscle cells were unresponsive compared to only 2 of the 16 endocardial cells. Purkinje cell RMP declined rapidly to the level of endocardial muscle during the first 10 minutes of superfusion with altered Tyrode's solution, but then slowed its rate of decline and was significantly greater than muscle after 15 minutes of superfusion with altered Tyrode's solution, although APA at this time was similar to endocardial muscle. After 30 minutes of washout, endocardial muscle tended to have greater RMP and APA than control, although the differences were not statistically significant. Epicardial muscle still had not returned to control after just 10 minutes of washout. No washout data were obtained for the papillary muscle preparations.

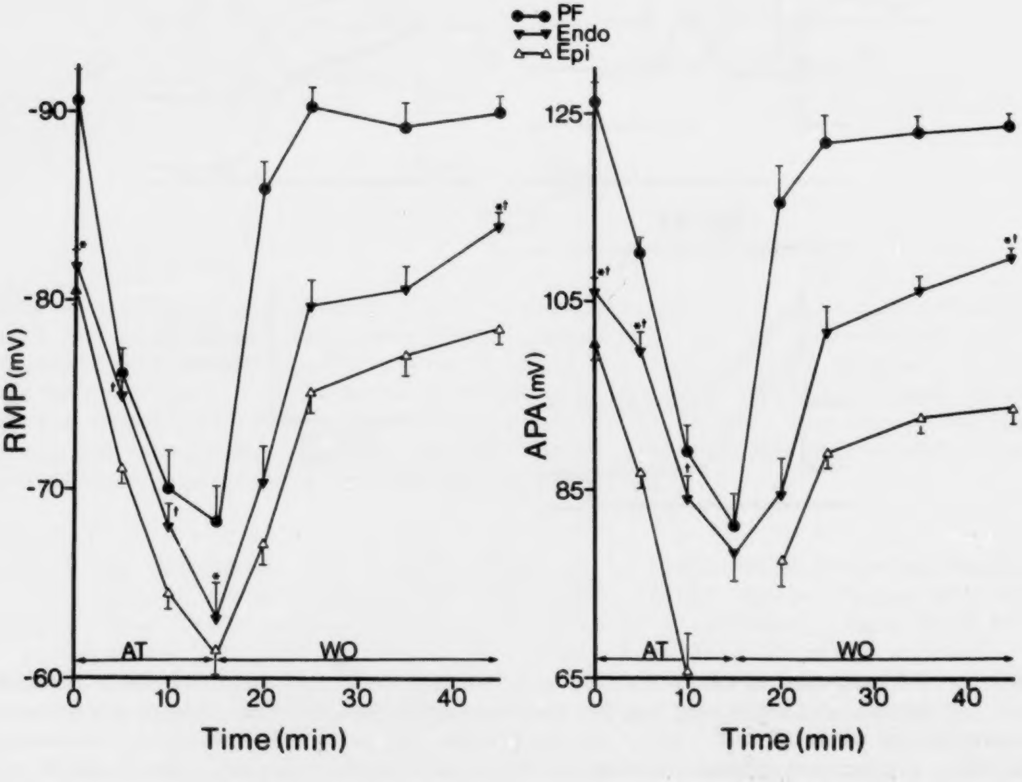

FIGURE 4. Changes in resting membrane potential (RMP) and action potential amplitude (APA) of Purkinje fiber (PF, n = 13) and endocardial (Endo, n = 16) and epicardial (Epi, n = 18) cells during superfusion with altered Tyrode's solution (AT) and washout (WO). Data are given as the mean ± SEM. * = Statistically significant difference between Endo and PF at $p < 0.05$. + = Statistically significant difference between Endo and Epi at $p < 0.05$. See text for discussion.

TABLE 1. Effects of Altered Tyrode's Solution (AT) On Action Potential Characteristics of Papillary Muscle

	C	5' AT	10' AT	15' AT
RMP (mV)	-82.3	-69.8	-63.2	-61.1
	± 0.9	± 1.2	± 0.9	± 0.7
APA (mV)	106.1	90.9	66.2	-----
	± 1.4	± 1.7	± 3.0	
APD_{50} (msec)	132.2	109.3	77.9	-----
	± 4.2	± 3.6	± 5.2	
APD_{90} (msec)	163.7	135.3	102.6	-----
	± 4.2	± 3.9	± 6.3	
dV/dt (V/sec)	209.9	140.6	26.2	-----
	±17.6	±14.7	± 4.2	
ACT (msec)	6.1	5.7	11.0	-----
	± 1.0	± 0.8	± 1.8	

All data are expressed as mean ± SEM. RMP = Resting membrane potential. APA = Action potential amplitude. APD = Action potential duration. dV/dt = Maximal rate of rise of phase zero. ACT = Activation time.

Changes in APD_{50} were equivalent to changes in APD_{90} during altered Tyrode's superfusion in endocardial muscle (figure 5). Epicardial changes in APD_{50} were greater than for endocardial muscle ($p < 0.05$), while APD_{90} changes were similar. Both APD_{50} and APD_{90} tended to shorten more in papillary muscle than in endocardium or epicardium. Shortening of APD_{50} in Purkinje fibers was more pronounced than APD_{90} reduction, resulting in similar APD_{50} for Purkinje fiber and endocardial muscle after 10 and 15 minutes exposure to altered Tyrode's solution, although APD_{90} for Purkinje fibers remained significantly longer than APD_{90} for endocardium at these times. The APD of endocardial and epicardial muscle types was slightly prolonged following 30 minutes of washout and the APD of Purkinje cells was prolonged to a greater extent, particularly APD_{50}.

Endocardial and epicardial muscle showed progressive decreases in dV/dt and corresponding increases in ACT with continued altered Tyrode's solution superfusion (figure 6). Despite a large reduction in dV/dt of Purkinje cells during superfusion with altered Tyrode's solution, ACT was not greatly affected. After 5 minutes of superfusion with altered Tyrode's solution there was a trend toward accelerated conduction in Purkinje fibers, i.e., activation times were less than the control, in spite of a reduction in dV/dt from 686.9 ± 43.8 to 541.2 ± 17.9. This was followed at 15 minutes by a modest, but significant ($p < 0.05$) increase in activation time that returned to control after only 5 minutes of washout. In papillary muscle there was also a trend toward accelerated conduction after 5 minutes of superfusion with altered Tyrode's solution, despite a 30 percent reduction of dV/dt. Further reductions in dV/dt were associated with increased ACT, but the increase in ACT tended to be less than in the endocardium or epicardium.

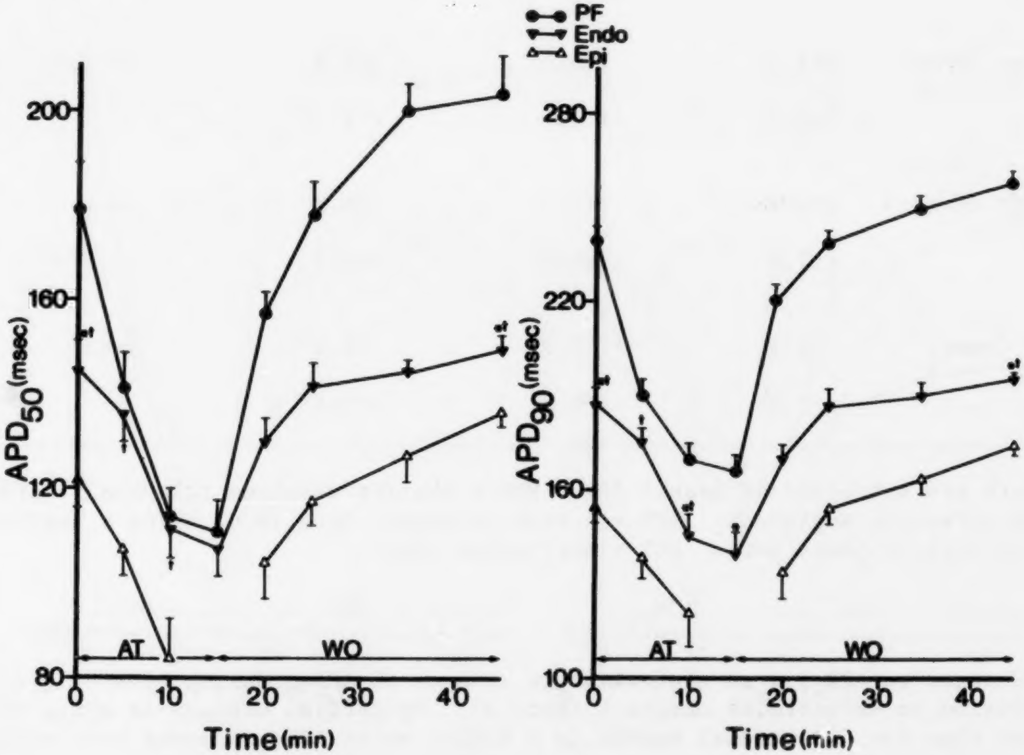

FIGURE 5. Changes in action potential duration at 50 percent of repolarization (APD_{50}) and at 90 percent of repolarization (APD_{90}) for Purkinje fiber (PF) and endocardial (Endo) and epicardial (Epi) cells during superfusion with altered Tyrode's solution (AT) and washout (WO). Data are given as the mean ± SEM. * and + are as given in figure 4.

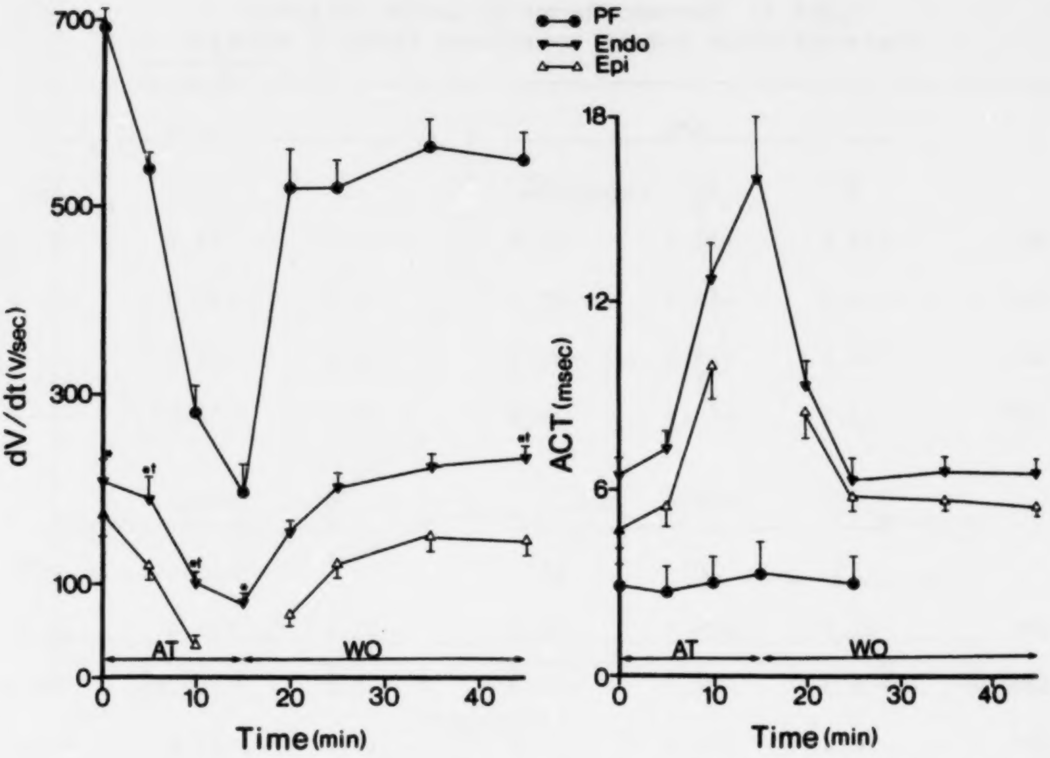

FIGURE 6. Changes in dV/dt and activation time (ACT) for Purkinje fiber (PF) and endocardial (Endo) and epicardial (Epi) cells during superfusion with altered Tyrode's solution (AT) and washout (WO). Data for ACT in PF are interelectrode conduction times. No statistical differences are given for ACT since the distances between stimulating and recording electrodes were not constant. Data are given as the mean ± SEM. * and + are as given in figure 4.

The average percentage change from control values in action potential characteristics following 5, 10, and 15 minutes of superfusion with altered Tyrode's solution for the four cell types is given in table 2. Percent changes in epicardial muscle generally exceeded those in endocardial muscle, but were less than changes in Purkinje fibers. Epicardium displayed a rapid reduction in dV/dt and an increase in ACT that exceeded those changes in endocardial muscle or in Purkinje fibers. Percent changes in papillary muscle were similar to those in epicardium, except for APD, which tended to shorten more in papillary muscle and, for ACT, which was less increased.

The relationship between time of superfusion with altered Tyrode's solution and washout, and the ERP/APD_{100} ratio is given in figure 7. In Purkinje cells, the ERP/APD_{100} ratio increased following 5 minutes of superfusion with altered Tyrode's solution but remained less than 1.0 during the entire 10-minute period of superfusion. Endocardial muscle showed more marked changes, resulting in postrepolarization refractoriness after 7 minutes of altered Tyrode's superfusion. Changes in ERP/APD_{100} ratio in epicardium were equivalent to those in

TABLE 2. Percent Change in Action Potential Characteristics Induced by Altered Tyrode's Solution

	RMP			APA		
	5'	10'	15'	5'	10'	15'
PF	-15.9	-22.7	-24.6	-12.3	-29.1	-35.2
ENM	- 8.3	-16.6	-22.7	- 5.8	-20.6	-25.8
EPI	-11.8	-19.6	-23.3	-13.8	-35.0	-----
PAP	-15.2	-23.2	-25.8	-14.3	-37.6	-----

	APD$_{50}$			APD$_{90}$		
	5'	10'	15'	5'	10'	15'
PF	-21.1	-36.0	-38.0	-21.4	-29.4	-31.1
ENM	- 6.3	-22.7	-25.9	- 6.2	-22.0	-24.6
EPI	-10.8	-31.4	-----	-10.8	-22.6	-----
PAP	-17.4	-41.1	-----	-17.3	-37.3	-----

	dV/dt			ACT		
	5'	10'	15'	5'	10'	15'
PF	-22.4	-59.3	-71.9	- 2.7	3.4	13.0
ENM	- 9.7	-50.8	-61.0	10.8	98.5	149.2
EPI	-30.8	-80.2	-----	14.9	114.9	-----
PAP	-33.0	-87.5	-----	- 6.2	80.9	-----

PF = Purkinje fiber. ENM = Endocardial muscle. EPI = Epicardial muscle. PAP = Papillary muscle. RMP = Resting membrane potential. APA = Action potential amplitude. APD = Action potential duration. dV/dt = Maximal rate of rise of phase zero. ACT = Activation time.

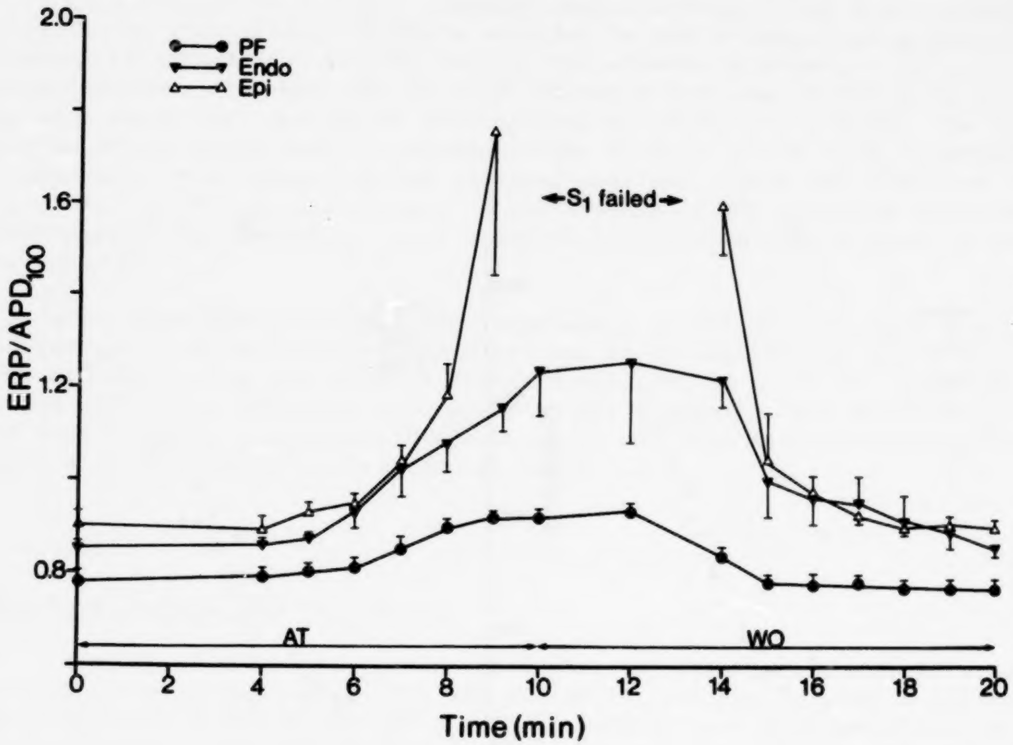

FIGURE 7. Effects of altered Tyrode's solution (AT) and washout (WO) on the ratio of effective refractory period and action potential duration measured at 100 percent of repolarization (ERP/APD$_{100}$) in Purkinje fiber (PF, n = 6) and endocardial (Endo, n = 5) and epicardial (Epi, n = 5) cells. Data are given as the mean ± SEM. All epicardial cells were unresponsive between 10 and 13 minutes. See text for discussion.

endocardium during the first 8 minutes of altered Tyrode's superfusion, but then the ERP/APD$_{100}$ ratio in epicardium increased rapidly. In these preparations all the epicardial cells became inexcitable after 10 minutes of superfusion with altered Tyrode's solution and were not excitable until after 4 minutes of washout.

Membrane Responsiveness

Representative examples of membrane responsiveness curves in endocardial, epicardial, and papillary muscle are shown in figure 8A during superfusion with normal Tyrode's solution. For TOP's more negative than approximately -75 mV, dV/dt's were similar for the three types of cells. At less negative TOP's, however, the magnitude of dV/dt decreased sharply for epicardium and papillary muscle compared to endocardium.

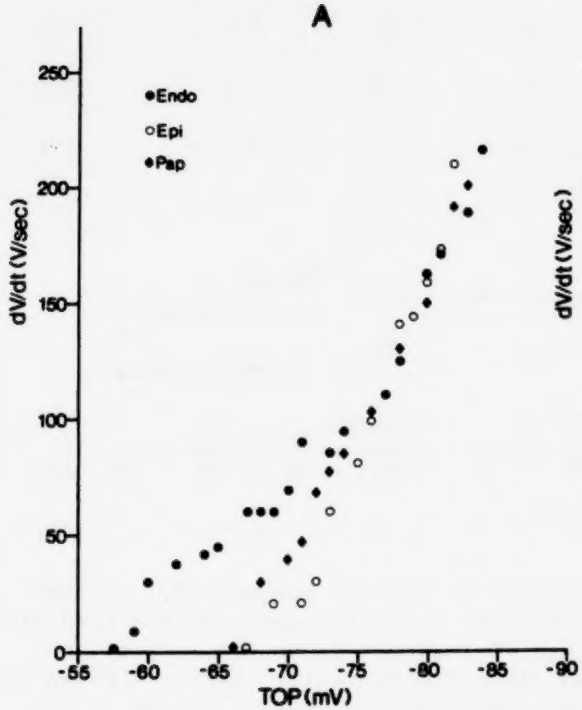

FIGURE 8. Relationship between take-off potential (TOP) and maximum rate of rise of phase zero (dV/dt) following premature stimulation during phases 3 and 4 (A) and following stimulation at a BCl of 500 msec during depolarization induced by superfusion with altered Tyrode's solution (B). Curves in A are representative examples for endocardial (Endo), epicardial (Epi), and papillary (Pap) muscle cells. Curves in B are constructed from the average dV/dt developed (n = 2-7) at any given TOP (corresponding to resting membrane potential) for the three cell types during the course of superfusion with altered Tyrode's solution. In A, Endo, Epi, and Pap have equivalent responsiveness at TOP's greater than -75 mV, but Epi and Pap responsiveness is less than Endo at less negative TOP's. Endo, Epi, and Pap also have similar responsiveness during superfusion with altered Tyrode's solution at TOP's greater than -68 mV, but Epi and Pap responsiveness is less than Endo at less negative TOP's.

We attempted to estimate membrane responsiveness after 5 to 6 minutes of superfusion with altered Tyrode's solution in two preparations of paired endocardial and epicardial pieces, despite the absence of steady-state conditions. In epicardium, the magnitude of dV/dt became a function of the S_1-S_2 interval, as well as of TOP, because of postrepolarization refractoriness. The latter caused action potentials to develop larger dV/dt's as the S_1-S_2 interval was lengthened, even though the TOP remained constant. This was also true for endocardium, but to a lesser extent. Rapid changes in RMP precluded measurement of membrane responsiveness at later times of superfusion with altered Tyrode's solution.

The relationship between TOP (equivalent to RMP in this case) and dV/dt for epicardial, endocardial, and papillary muscle during control and at various times during superfusion with altered Tyrode's solution is given in figure 8B. For the three cell types dV/dt was equivalent at TOP's greater than approximately -68 mV. At less negative potentials, however, dV/dt was less in epicardial and papillary muscle cells compared to endocardial cells.

DISCUSSION

New Observations From This Study

Results of this study demonstrate that (a) superfusion with altered Tyrode's solution containing $[K^+]_o$ - 8.0 mM, pH - 6.85, and pO_2 < 50 mm Hg affected activation time, excitability, and action potentials to a greater degree in the epicardium than in the endocardium or in Purkinje fibers; papillary muscle displayed changes in excitability and action potentials that were similar to epicardium; (b) during continued superfusion with altered Tyrode's solution, Purkinje fibers, and to a lesser degree, endocardium, underwent partial recovery; (c) less marked changes occurred in epicardium and endocardium during the second exposure to altered Tyrode's solution than during the first.

Superfusion With Altered Tyrode's Solution

Increases in epicardial activation time during superfusion with altered Tyrode's solution in vitro may be similar to the delayed activation recorded in epicardial electrograms during acute myocardial ischemia in vivo. The fragmentation of electrogram recordings and local "continuous" electrical activity that resulted from desynchronous activation in vivo may reflect the presence of graded epicardial responses or different degrees of depression in the plateau phases of epicardial action potential, similar to those reported by Downar et al. (6-7).

The greater sensitivity of epicardial versus endocardial muscle to superfusion with altered Tyrode's solution may be due to intrinsic differences in membrane responsiveness, particularly as the membrane potential is reduced. The APD and dV/dt tended to be slightly lower in control for epicardial muscle compared to endocardial muscle, although only the difference in APA was statistically significant. Membrane responsiveness at TOP's less than -75 mV was attenuated during control in epicardium compared to endocardium. During 15 minutes of superfusion with altered Tyrode's solution, changes in RMP were parallel for epicardium and endocardium, yet changes in APA, dV/dt, and ACT, as well as loss of excitability, tended to be greater in epicardium. Increasing stimulus intensity or duration transiently restored epicardial responsiveness, but further increases in excitability threshold rapidly ensued and epicardial muscle became inexcitable.

The differences in membrane responsiveness between epicardium and endocardium during control or exposure to altered Tyrode's solution are probably due, at least in part, to the presence of Purkinje cells in the endocardial layers. Endocardial muscle cells not in contact with Purkinje cells (i.e., papillary muscle tip cells) had membrane responsiveness curves and changes of APA and dV/dt and loss of excitability during superfusion with altered Tyrode's solution that were similar to epicardium, despite control APA, RMP, and dV/dt values that approximated more closely those of endocardium than epicardium. The higher incidence of accelerated conduction in papillary muscle during the first 5 minutes of superfusion with altered Tyrode's solution (all types of cells occasionally displayed accelerated conduction) resulted in less conduction delay at 10 minutes of superfusion in papillary muscle than in endocardium or epicardium, yet approximately the same percentage of papillary and epicardial muscle cells became unresponsive at 10 and 15 minutes of superfusion.

Partial Recovery

We observed partial recovery of Purkinje cells and, to a lesser extent, endocardial muscle cells, during 20-60 minutes of altered Tyrode's superfusion. Recovery of epicardial or subepicardial cardiac electrical activity during this time period has been described following acute myocardial ischemia in vivo (3,7, 11) and has been ascribed to increases in collateral flow or diffusion of metabolites such as K^+ from the ischemic zone extracellular space. Our data indicate that improved cellular electrical activity of endocardium and Purkinje fibers can occur in an environment of constant hypoxia, hyperkalemia, and acidosis, even without exogenous glucose and free fatty acids.

Although we did not observe significant recovery of electrical activity in separated pieces of epicardium, endocardial improvement could possibly contribute to the recovery of epicardial activity during myocardial ischemia in vivo. This is supported by the observation that papillary muscle cells showed no evidence of recovery in preparations where only the tip of the muscle was used, but recovery was observed in two preparations that included a basal area of muscle previously connected to a false tendon. Simultaneous recordings of action potentials from the tip and basilar areas indicated that recovery of the basilar cell preceded the return of excitability in the tip that had been previously unresponsive.

Since maintenance of simultaneous microelectrode impalements in epicardial and endocardial pieces for at least 60 minutes was difficult, accurately quantifying the differences in electrical response of these tissues to repeated superfusion with altered Tyrode's solution was not possible. The data in three preparations in which impalements were maintained throughout successive superfusions suggest, however, that less marked changes occurred during the second exposure to altered Tyrode's solution than during the first exposure. This observation may represent a cellular correlate to the observation that the initial occlusion of a coronary artery produces more severe electrical changes than subsequent occlusions (1,3,12). Superfusion with elevated potassium in vitro, however, may have merely replenished potassium losses incurred during dissection and equilibration of the preparation (13), resulting in more negative resting potentials and a diminished response to the depolarizing effects of subsequent superfusion with altered Tyrode's solution.

CONCLUSIONS

From these experiments we conclude that the resistance of Purkinje fibers to the effects of hyperkalemia, acidosis, and hypoxia may contribute to the preservation of endocardial electrical activity. Applying this conclusion to the in vivo model during acute myocardial ischemia obviates the need to invoke the effects of Thebesian blood flow, superfusion by cavity blood, or greater capacity of subendocardium for anaerobic glycolysis, although these phenomena may still play a role in vivo. While it is possible that endocardium may be intrinsically more resistant than epicardium to the effects of ischemia, the similar response of papillary muscle tip and epicardium make this conclusion less likely.

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DECREASE IN FAST INWARD SODIUM CURRENT AS A POSSIBLE CAUSE OF THE ANTIARRHYTHMIC EFFECT OF ETHMOZIN, MEXITIL, AND LIDOCAINE IN THE LATE STAGE OF EXPERIMENTAL MYOCARDIAL INFARCTION

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ABSTRACT. In dogs 24 hours after occlusion of a coronary artery (i.e., late stage of experimental myocardial infarction), intravenous administration of a specific blocker of the fast sodium channels, i.e., tetrodotoxin, at 2.2 ± 0.3 μg/kg (n = 8), resulted in elimination of more than 50 percent of ventricular arrhythmias. A dose of tetrodotoxin at one-half the threshold dose (i.e., subthreshold dose) had no antiarrhythmic effect. Simultaneous administration of a subthreshold dose of tetrodotoxin (1.1 ± 0.2 μg/kg and a subthreshold dose of ethmozin (0.9 ± 0.1 mg/kg), mexitil (1.6 ± 0.4 mg/kg), or lidocaine (3.8 ± 1.4 mg/kg) resulted in elimination of arrhythmia in the late stages of experimental myocardial infarction. Thus, tetrodotoxin potentiated the antiarrhythmic effect of the other preparations used. This fact indicated that the antiarrhythmic effect of these substances results from their capability to decrease the fast inward sodium current.

This conclusion was confirmed in special experiments in which the antiarrhythmic activity of two phenothiazines--ethmozin and its diethylamine analog (ethmozin DAA)--was compared with the capability of these preparations to influence transmembrane ion currents, studied by fixation of the potential on atrial frog fibers. The duration of the antiarrhythmic effect of ethmozin DAA was several times greater than that of ethmozin. Both phenothiazines decreased the fast inward sodium current, which was not restored as the ethmozin DAA was washed out, but was fully restored as the ethmozin was washed out. Neither of these phenothiazines changed the outward currents; ethmozin increased and ethmozin DAA decreased the slow

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inward current. These data agree with the hypothesis that the cause of the antiarrhythmic effect of drugs in the late stage of experimental myocardial infarction is a decrease in the fast inward sodium current.

INTRODUCTION

Cardiac rhythm disorders in the late stage of experimental myocardial infarction (24 hours after occlusion of a coronary artery) can be eliminated by antiarrhythmic preparations such as ethmozin, mexitil, lidocaine, and others (1-7). The overall effect of these preparations on the myocardial fibers is one of slowing the rate of rise of the transmembrane action potential (4-6,8,9), thus indicating a decrease in the fast inward sodium current. Therefore, it has been suggested that the relief of arrhythmias in the late stage of experimental infarction is related to the effect of these antiarrhythmic preparations on the fast inward sodium current in the myocardial fibers.

To test this hypothesis, we conducted a study on dogs 24 hours after occlusion of a coronary artery. We investigated (a) the antiarrhythmic activity of tetrodotoxin, a specific blocker of the fast sodium current (10); (b) the possibility of potentiating the antiarrhythmic effect of ethmozin, mexitil, and lidocaine by using tetrodotoxin; and (c) the antiarrhythmic activity of ethmozin and the diethylamine analog of ethmozin--ethmozin DAA (11). Ethmozin DAA was synthesized at the Institute of Pharmacology, USSR Academy of Medical Sciences. In addition, special experiments involving recording of the potential from isolated frog atrial trabeculae were used to compare the effect of ethmozin and ethmozin DAA on the fast and slow inward and outward transmembrane ion currents.

MATERIALS AND METHODS

Experiments were performed on random-bred dogs on both sexes weighing 8-20 kg. The animals were put under nembutal narcosis (30-35 mg/kg intravenously) and artificially respirated under sterile conditions. The thoracic cavity was then entered through the fourth intercostal space. The pericardium was dissected and the left descending branch of the coronary artery was separated from the surrounding tissue at a distance of 1-2 cm from the auricula of the left atrium. Occlusion of the artery was performed in two stages by Harris' method (12). A polyethylene catheter was inserted into the external jugular vein, passed beneath the skin, and attached to the neck for later intravenous administration of the preparations to be tested. The thorax was sutured and the animals were returned to natural respiration. Twenty-two to 24 hours after the operation, the electrocardiogram (ECG) was recorded in the second standard lead and, if arrhythmia was present, the substances to be studied were administered intravenously.

In the first series of experiments (on eight dogs), we determined the capacity of tetrodotoxin to depress rhythm disorders in the late stage of infarction. The initial dose of tetrodotoxin was 0.5 μg/kg; the effects of 1, 2, and 3 μg/kg were then tested.

The effective threshold dose was considered to be the minimum dose which had a clear antiarrhythmic effect, i.e., by decreasing the number of extrasystoles by more than 50 percent.

In the second series of experiments (on 10 dogs), we studied the capacity of tetrodotoxin to potentiate the effect of ethmozin. For this purpose, the method described above was used to determine the threshold dose of tetrodotoxin in each experiment. To determine the threshold dose of ethmozin, we administered 2 mg/kg of the preparation intravenously. If there was a clear antiarrhythmic effect, the dose was cut in half; if there was no effect, the dose of ethmozin was doubled. This procedure was continued until we determined the minimum dose of ethmozin capable of causing a clear antiarrhythmic effect; this was called the threshold dose. Doses of tetrodotoxin and ethmozin at half the threshold levels had practically no effect on the arrhythmias. These doses were considered subthreshold. After we determined the threshold and subthreshold doses for ethmozin and tetrodotoxin, we tested the antiarrhythmic effect of a mixture of subthreshold doses of these substances.

In the third and fourth series of experiments, following a methodology similar to that used in the second series, we studied the capacity of tetrodotoxin to potentiate the antiarrhythmic effect of mexitil (in seven dogs) and lidocaine (in five dogs).

In all experiments, the necessary quantities of the substances to be tested were dissolved in 5 ml of saline and administered over a time of 1 minute. The intervals between administration of the substances were at least 1 hour. The initial level of arrhythmia usually returned 15-40 minutes after administration of the threshold dose of the substance. In order to decrease the central effect of tetrodotoxin and the arrhythmic preparations studied, the animals received 100 mg/kg chloralose intravenously. The administration of chloralose had practically no effect on the arrhythmias. The ECG's were recorded before administration, during administration, and during the entire period of action of the substances. The total number of ventricular complexes per minute was counted, as well as the percentage of ectopic ventricular excitations.

In the fifth series of experiments, we compared the antiarrhythmic activity of ethmozin in three doses (1, 2, and 3 mg/kg) (in 19 dogs), as well as the antiarrhythmic effect of ethmozin DAA, also in three doses (0.5, 1.0, and 1.5 mg/kg) (in 18 dogs). In this series of experiments, the doses of the substances were administered once, and after the antiarrhythmic effect was evaluated, the animals were not used in subsequent experiments.

Ion currents were recorded on isolated atrial trabeculae of Rana temporaria and Rana catesbeiana. An atrial trabecula 75-120 μm in diameter and 3-5 mm in length was removed from the atrium and placed in a perfusion chamber with a double saccharose bridge (9). Low-resistance (less than 5 kΩ) extracellular Ag-AgCl electrodes with agar bridges and a Dagan (USA) electronic circuit were used to record the action potential and current. The output voltage of the amplifier, with negative feedback used to record the potential, was ± 90 V; the gain factor was 25,000. The test section of the chamber (200 μm wide) was perfused with Ringer's solution of the following composition (in millimoles): NaCl 114, KCl 2.7, $CaCl_2$ 1.8, tris-HCl 10, glucose 6.5, pH 7.5-7.6. All experiments were performed at 20-22° C. The fast inward sodium current was recorded in a control, and under the influence of ethmozin ($5 \cdot 10^{-6}$ g/ml) and ethmozin DAA ($5 \cdot 10^{-6}$ g/ml) in the presence of a D 600 preparation, in order to suppress the slow inward current. The effect of phenothiazines on the slow inward and outward currents was studied during perfusion with Ringer's solution containing tetrodotoxin ($6 \cdot 10^{-7}$ g/ml), or tetrodotoxin ($6 \cdot 10^{-7}$ g/ml) and preparation D 600 ($1 \cdot 10^{-6}$ g/ml).

RESULTS

Antiarrhythmic Effect of Tetrodotoxin in the Late Stage of Experimental Myocardial Infarction

The results of a typical experiment are shown in figure 1. This figure shows that 24 hours after occlusion of the coronary artery, ventricular

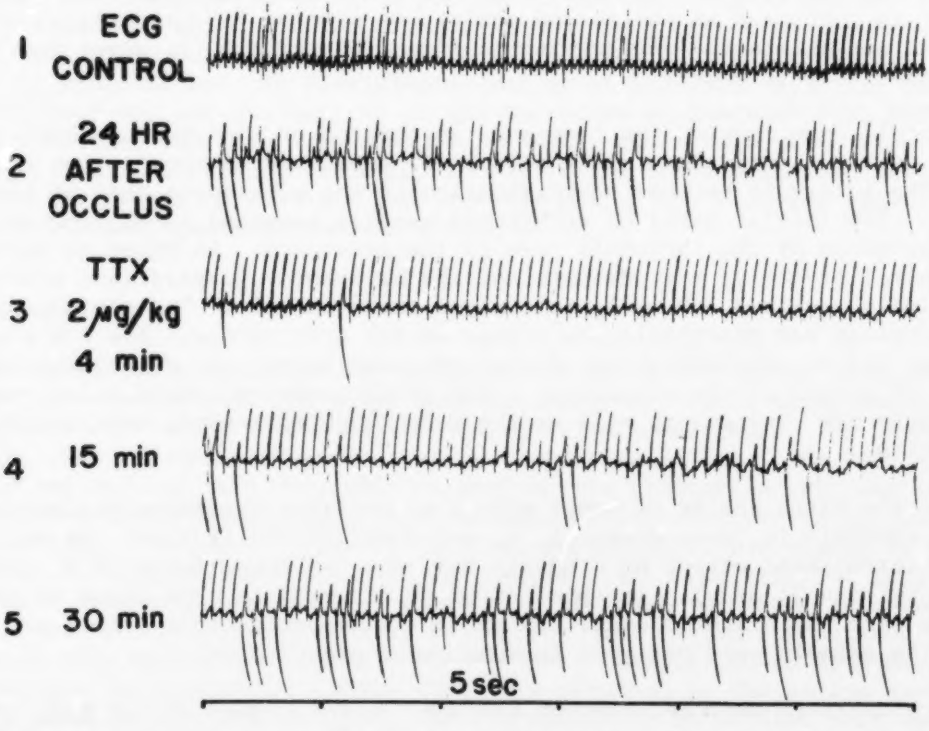

FIGURE 1. Antiarrhythmic effect of tetrodotoxin (TTX) in the late stage of experimental myocardial infarction. 1 = ECG before occlusion of coronary artery. 2 = ECG 24 hours after occlusion of coronary artery. 3,4,5 = ECG after 4, 15, and 30 minutes following administration of TTX intravenously at 2 μg/kg.

extrasystoles were present (curve 2). During the fourth minute after completion of administration of 2 μg/kg of tetrodotoxin, the sinus rhythm was almost completely restored (curve 3). During the fifteenth minute, a significant number of ectopic ventricular excitations appeared (curve 4), and by 30 minutes the initial level of arrhythmia had been completely restored (curve 5).

The summary data from eight experiments are presented in table 1. As can be seen from the table, the threshold dose of tetrodotoxin in various experiments ranged from 0.5 to 3.0 μg/kg. Administration of threshold doses significantly decreased (by a factor averaging more than 10) the percentage of ventricular extrasystoles ($p < 0.001$). In three animals after administration of tetrodotoxin, sinus rhythm was completely restored. These data indicate that this specific fast sodium channel blocker suppresses ventricular arrhythmias arising 24 hours after occlusion of a coronary artery.

TABLE 1. Decrease in the Number of Ectopic Ventricular Excitations (% VE) Upon Intravenous Administration of Tetrodotoxin in the Late Stage of Experimental Myocardial Infarction

No.	Dose (μg/kg)	Control		Maximum effect		Time* (min)	Duration** (min)
		Total rhythm	% VE	Total rhythm	% VE		
1	3	196	81	122	11	4	19
2	3	116	67	134	7	5	12
3	1	130	100	140	3	3	8
4	3	186	87	148	9	12	31
5	0.5	164	74	152	0	2	25
6	3	106	69	126	0	3	51
7	2	166	71	166	21	3	26
8	2	134	100	148	0	3	5
M	2.2	149.8	81.1	142.0	6.4	4.4	22.1
± m	± 0.3	± 11.6	± 4.7	± 5.1	± 2.6	± 1.1	± 5.3

*Time required to achieve maximum effect after completion of administration of tetrodotoxin.

**Time required for effect to drop to 50 percent of maximum effect.

Potentiation of the Antiarrhythmic Effect of Ethmozin, Mexitil, and Lidocaine With Tetrodotoxin

Figure 2 shows the results of a typical experiment determining the threshold and subthreshold doses of ethmozin and tetrodotoxin, and then testing the combined effect of subthreshold doses of both preparations. Curve 2 shows the ventricular arrhythmias, on the background of which the experiment was performed.

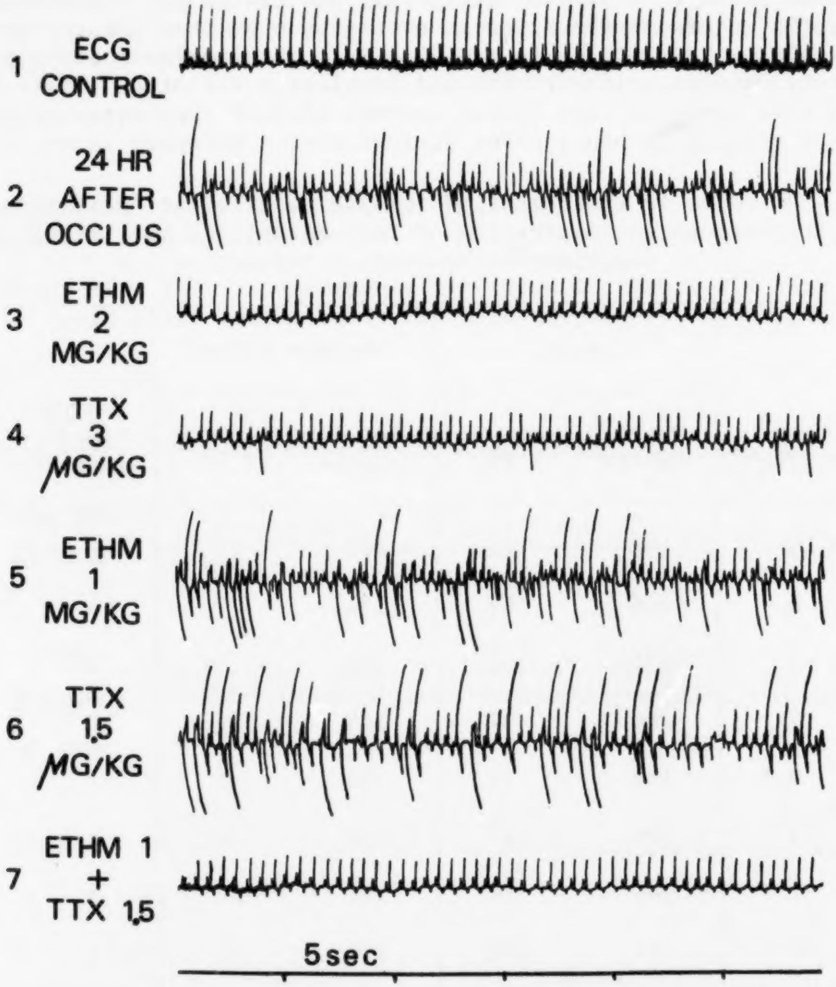

FIGURE 2. Potentiation of antiarrhythmic effect of ethmozin (ETHM) in the late stage of experimental myocardial infarction by tetrodotoxin (TTX). 1 = ECG before occlusion of coronary artery. 2 = ECG 24 hours after occlusion of coronary artery. 3,5 = Second minute of effect of 2 mg/kg and 1 mg/kg ethmozin. 4,6 = Third minute of effect of 3 μg/kg and 1.5 μg/kg tetrodotoxin. 7 = Second minute of effect of mixture of subthreshold doses of the two substances: 1 mg/kg ethmozin and 1.5 μg/kg tetrodotoxin.

Ethmozin at 2 mg/kg fully normalized cardiac rhythm (curve 3). The number of ventricular extrasystoles was significantly reduced by administration of 3 μg/kg tetrodotoxin (curve 4). These substances at half of these concentrations had no antiarrhythmic effect (curves 5 and 6). Administration of the mixture of subthreshold doses of ethmozin and tetrodotoxin completely normalized cardiac rhythm (curve 7).

The summary results of 10 experiments involving separate administration of threshold and joint administration of subthreshold doses of ethmozin and tetrodotoxin are presented in table 2, section 1. As can be seen from the table, administration of ethmozin significantly decreased the percentage of ventricular extrasystoles; the maximum effect was observed 1-2 minutes after administration of the preparation. The percentage of ventricular extrasystoles was greatly decreased after administration of threshold doses of tetrodotoxin as well; however, the time required to reach the maximum effect averaged 2.5 minutes more than for ethmozin ($p < 0.05$). Administration of a mixture of subthreshold doses of ethmozin and tetrodotoxin decreased the number of ventricular extrasystoles to equal that achieved by separate administration of the threshold doses of these substances.

Table 2, sections 2 and 3, present summary data from experiments involving separate administration of threshold and joint administration of subthreshold doses of mexitil and tetrodotoxin, as well as lidocaine and tetrodotoxin. As can be seen from the table, administration of a mixture of subthreshold doses of mexitil and tetrodotoxin or lidocaine and tetrodotoxin decreased the number of ventricular extrasystoles to equal that achieved by separate administration of threshold doses of these substances.

Antiarrhythmic Effect of Ethmozin and Ethmozin DAA in the Late Stage of Experimental Myocardial Infarction

Ethmozin at 2 mg/kg caused complete suppression of ectopic ventricular rhythm in most experiments (figure 3A). The preparation was effective within 1 minute and its effect continued for 10-15 minutes, after which ventricular extrasystoles reappeared. The short time of action of ethmozin has also been reported in other works (1,2,4). Ethmozin at 3 mg/kg did not completely relieve arrhythmias, and in some animals administration of the preparation in this dose was followed by aggravation of arrhythmias.

Since the antiarrhythmic activity of ethmozin DAA is twice as strong as that of ethmozin (11), ethmozin DAA was tested in doses of 0.5-1.5 mg/kg. In two out of six animals, ethmozin DAA at 0.5 mg/kg completely eliminated rhythm disorders, and the antiarrhythmic effect continued for more than 1 hour. The time of onset of the antiarrhythmic effect of ethmozin DAA was about 1 minute, as in the case of ethmozin (figure 3B). At a dose of 1.5 mg/kg, ethmozin DAA completely suppressed the arrhythmias which did not reappear during the subsequent 60 minutes of observation. Thus, in comparison with ethmozin, ethmozin DAA manifests its antiarrhythmic effect at lower concentrations and over a much longer period of time.

TABLE 2. Potentiation of the Antiarrhythmic Effect of Ethmozin, Mexitil, and Lidocaine by Tetrodotoxin. Decrease in Number of Ectopic Ventricular Excitations (% VE) With Separate Administration of Threshold (A and B) and Combined Administration of Subthreshold (C) Doses of Tetrodotoxin and the Corresponding Antiarrhythmic Substances

	Control		Maximum effect			
Dose	Total rhythm	% VE	Total rhythm	% VE	Time* (min)	Duration** (min)
			1. Ethmozin (n = 10)			
A. Ethmozin (mg/kg)						
1.8 ± 0.1	152.2 ± 12.1	78.3 ± 4.3	138.8 ± 5.7	9.4 ± 3.1	1.7 ± 0.3	14.4 ± 3.8
B. Tetrodotoxin (μg/kg)						
2.3 ± 0.3	145.8 ± 9.7	80.0 ± 4.5	139.8 ± 4.3	6.6 ± 2.3	4.2 ± 0.9	22.0 ± 4.2
C. Ethmozin (mg/kg) + tetrodotoxin (μg/kg)						
0.9 ± 0.1 + 1.1 ± 0.2	147.2 ± 8.5	78.3 ± 3.2	129.7 ± 5.9	8.1 ± 1.9	2.0 ± 0.3	11.9 ± 2.6
			2. Mexitil (n = 7)			
A. Mexitil (mg/kg)						
3.1 ± 0.9	157.8 ± 12.3	71.6 ± 7.4	143.9 ± 8.9	11.9 ± 5.3	3.9 ± 1.1	16.1 ± 3.1
B. Tetrodotoxin (μg/kg)						
2.1 ± 0.4	155.1 ± 7.8	82.1 ± 6.0	155.1 ± 8.0	9.0 ± 3.4	4.9 ± 1.2	20.7 ± 4.0
C. Mexitil (mg/kg) + tetrodotoxin (μg/kg)						
1.6 ± 0.4 + 1.1 ± 0.2	160.0 ± 8.9	71.7 ± 7.8	152.4 ± 7.2	8.8 ± 4.0	2.9 ± 0.5	21.0 ± 5.1
			3. Lidocaine (n = 5)			
A. Lidocaine (mg/kg)						
7.6 ± 2.7	142.2 ± 11.7	84.6 ± 9.6	120.8 ± 6.2	13.0 ± 8.8	2.6 ± 1.1	13.2 ± 2.9
B. Tetrodotoxin (μg/kg)						
3.0 ± 0.8	150.2 ± 11.5	85.4 ± 7.0	136.8 ± 2.1	7.8 ± 6.0	4.4 ± 1.4	17.2 ± 3.1
C. Lidocaine (mg/kg) + tetrodotoxin (μg/kg)						
3.8 ± 1.4 + 1.5 ± 0.4	148.8 ± 13.6	83.2 ± 5.8	126.0 ± 3.0	3.0 ± 3.0	2.4 ± 0.5	12.0 ± 1.9

*Time required to achieve maximum antiarrhythmic effect after completion of administration of substance.
**Time required for effect to drop to 50 percent of maximum effect.
M ± m. The drop in % VE in all cases was statistically significant ($p < 0.001$).

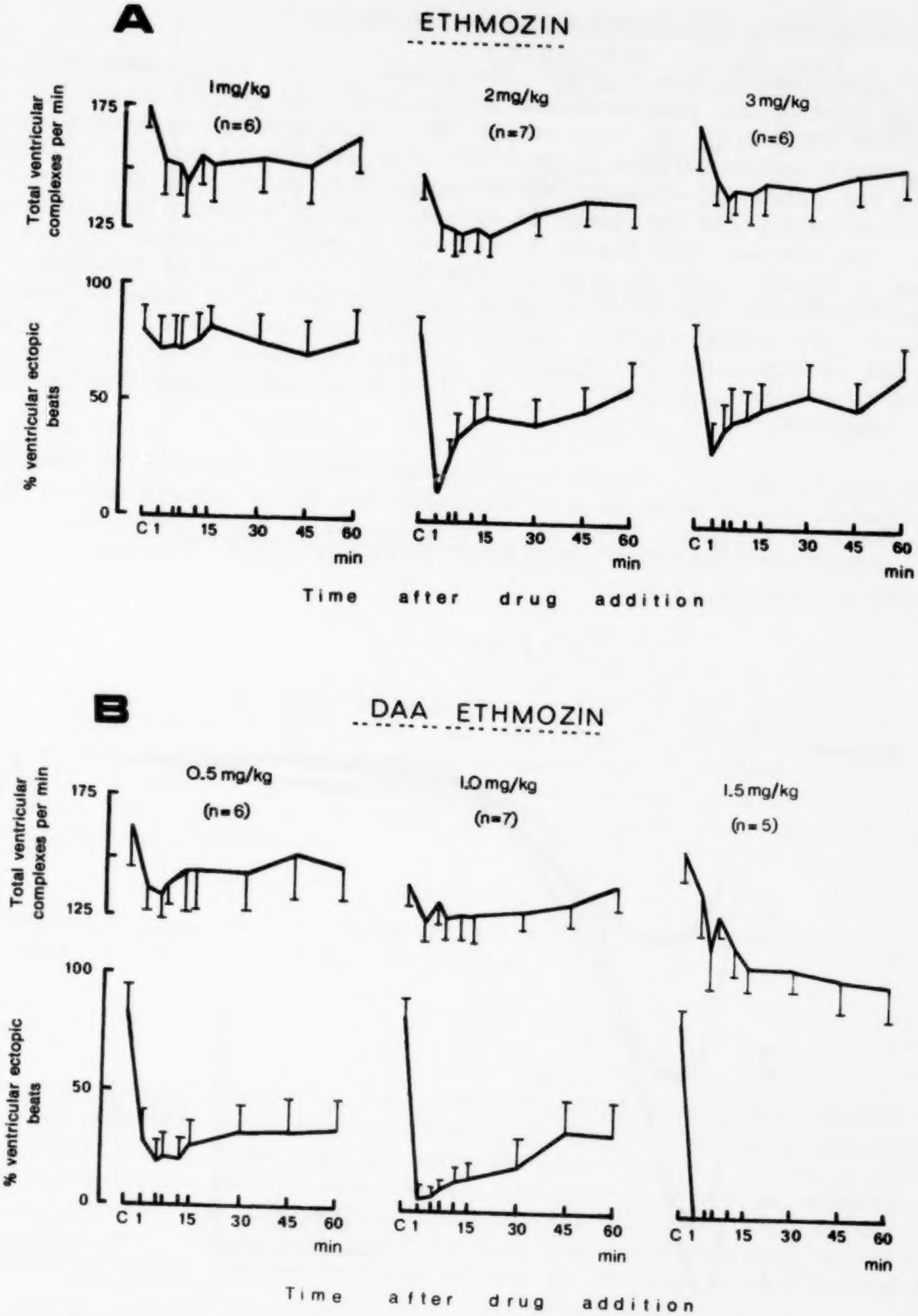

FIGURE 3. Influence of ethmozin in doses of 1, 2, and 3 mg/kg (A) and ethmozin DAA in doses of 0.5, 1.0, and 1.5 mg/kg (B) on ventricular rhythm disorders in the late stage of experimental myocardial infarction. In top of figure, each graph shows concentrations of substances in A and B. Parentheses show number of experiments. C = Control. Graphs show M ± σ.

Influence of Ethmozin and Ethmozin DAA on Ion Currents

Fast inward sodium ion current. We showed earlier that ethmozin decreases the rate of rise of the transmembrane action potential and decreases the fast inward sodium current in the frog atrium (9). Ethmozin DAA also decreases the fast inward sodium current (figure 4). The changes in maximum sodium current under the effect of ethmozin and ethmozin DAA, as well as the dynamics of the sodium current in specimens in normal Ringer's solution are shown in figure 5. One can see from figure 5 that the decrease in sodium current under the effect of ethmozin DAA is greater than under the effect of ethmozin. As the Ringer's solution continues to bathe the specimen after ethmozin has been administered, the sodium current returns to its previous level, while after ethmozin DAA is administered the sodium current continues to drop. Thus, in comparison to ethmozin, ethmozin DAA has a stronger and longer-lasting effect on sodium currents.

Slow inward current. Ethmozin caused an increase in the slow inward current in each of six experiments (figures 6 and 8), whereas ethmozin DAA caused a decrease (figures 7 and 8). The increase in slow inward current under the effect of ethmozin is explained by the positive ionotropic effect of this preparation (9,13).

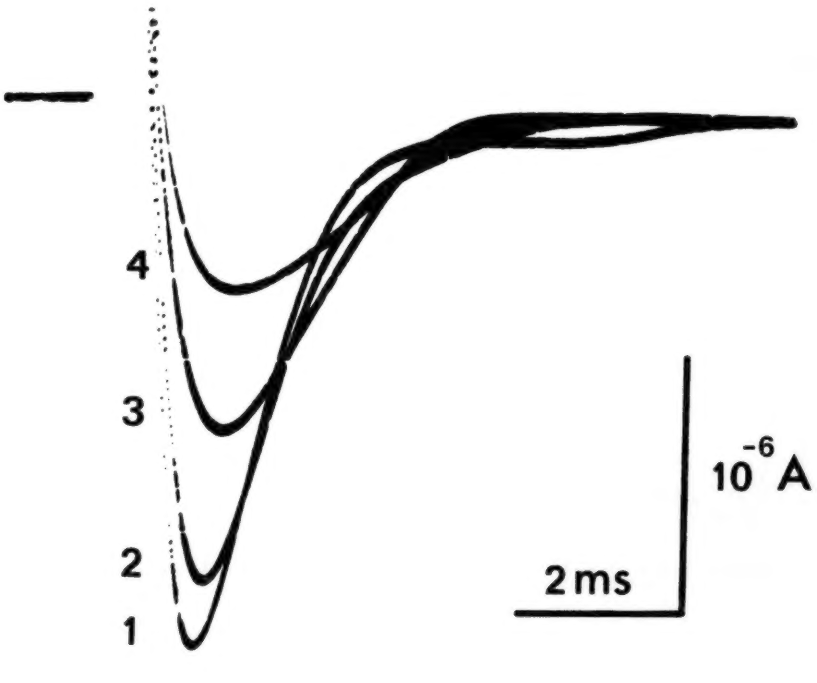

FIGURE 4. Effect of ethmozin DAA ($2 \cdot 10^{-5}$ g/ml) on the maximum value of the fast inward sodium current recorded in the atrial trabeculum of the frog with depolarization of the membrane to 40 mV from the resting potential. 1 = Control. 2,3,4 = After 3, 7, and 10 minutes of action of ethmozin DAA.

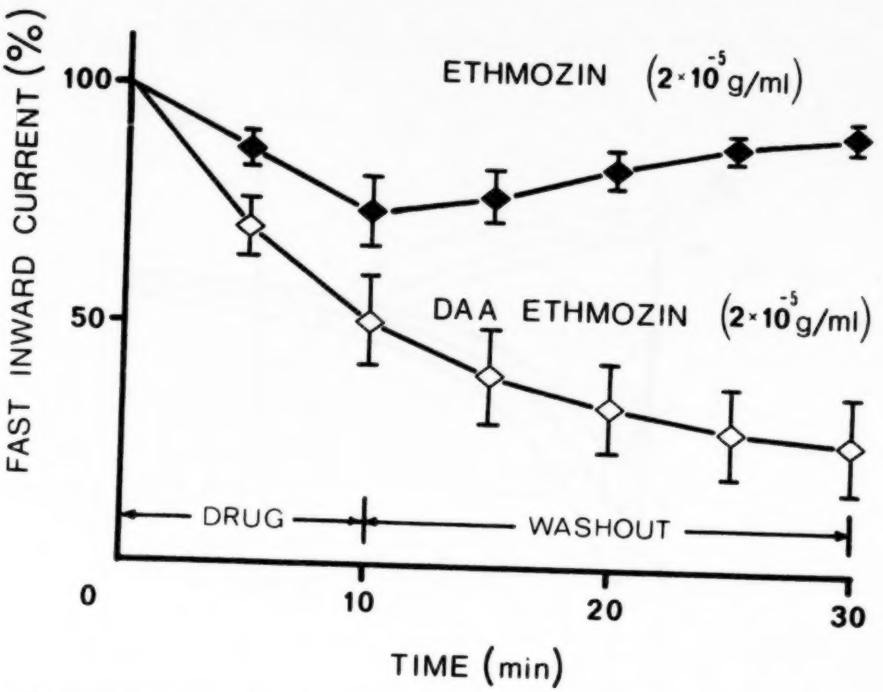

FIGURE 5. Change in the fast inward sodium current in the atrial trabecula of the frog with ethmozin ($5 \cdot 10^{-6}$ g/ml) and with ethmozin DAA ($5 \cdot 10^{-6}$ g/ml), and dynamics of the sodium current as the substances are washed out with Ringer's solution not containing phenothiazines. Values for the currents are expressed in percent of the initial value, taken as 100 percent. The graphs show M ± σ obtained from nine experiments.

Outward current. Neither ethmozin nor ethmozin DAA caused any significant changes in outward currents (figure 9).

DISCUSSION

The experiments showed that tetrodotoxin, which blocks fast inward sodium current, has a strong antiarrhythmic effect in the late stage of experimental myocardial infarction. The time of onset and duration of the antiarrhythmic effect coincide approximately with the antiarrhythmic effect of ethmozin, mexitil, and lidocaine; tetrodotoxin potentiated the antiarrhythmic effect of these preparations. These data indicate that the antiarrhythmic effect of the substances studied is related to their effect on the fast inward sodium current.

The ability of ethmozin and mexitil to influence the fast inward sodium current has been well documented in a number of experimental works (5,9,13). However, the effect of lidocaine on the excitability of the myocardium has been described in contradictory ways in the literature. In early studies, no clearly expressed effect of lidocaine on the rate of transmission of excitation, refractoriness, or other parameters characterizing excitability of the

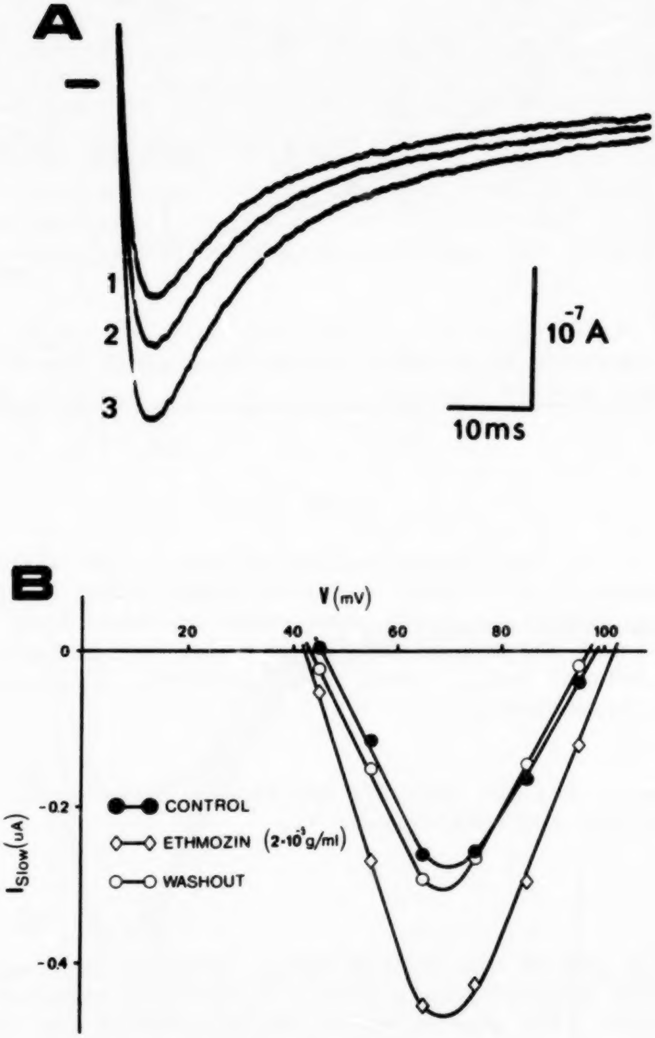

FIGURE 6. Effect of ethmozin ($2\cdot10^{-5}$ g/ml) on the slow inward current in frog atrial trabecula. A: 1 = Maximum value of the slow current obtained in the control with depolarization 70 mV from the resting potential. 2,3 = 5 and 10 minutes ethmozin. B: Volt-ampere curves of slowly entering current in the control, after 10 minutes of ethmozin, and after 30 minutes washing with a normal Ringer's solution containing no ethmozin.

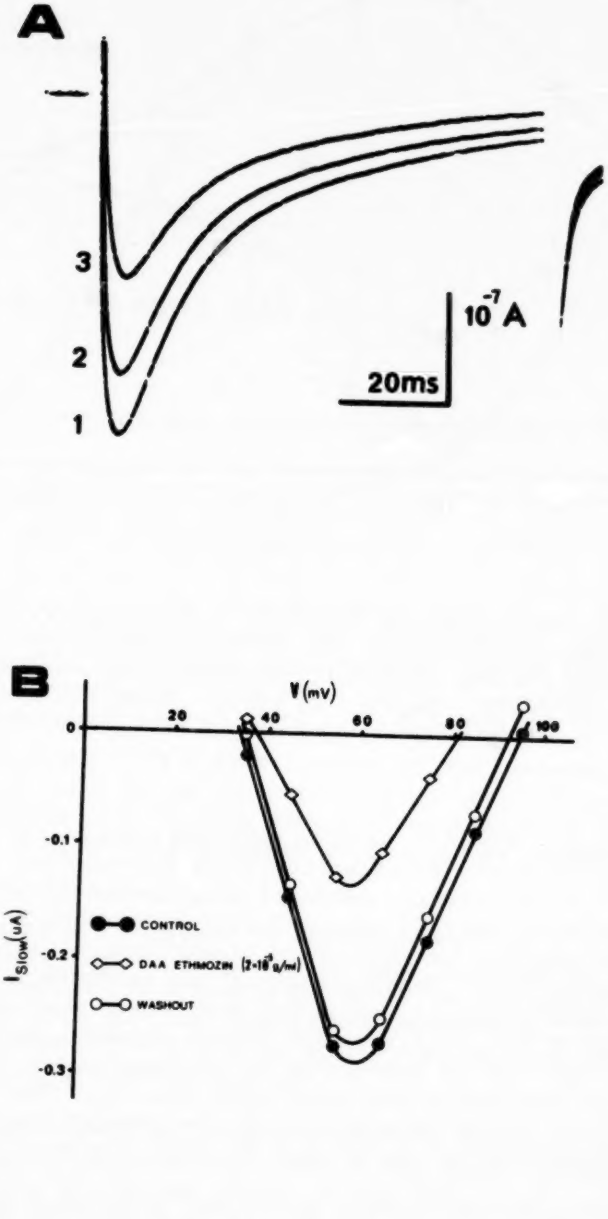

FIGURE 7. Effect of ethmozin DAA ($2 \cdot 10^{-5}$ g/ml) on the slow inward current in frog atrial trabecula. A: 1 = Maximum value of the slow current obtained in the control with depolarization of 74 mV from the resting potential. 2,3 = 5 and 10 minutes ethmozin DAA. B: Volt-ampere curves of the slow inward current in the control, after 10 minutes of ethmozin DAA, and 30 minutes of washing with normal Ringer's solution containing no ethmozin DAA.

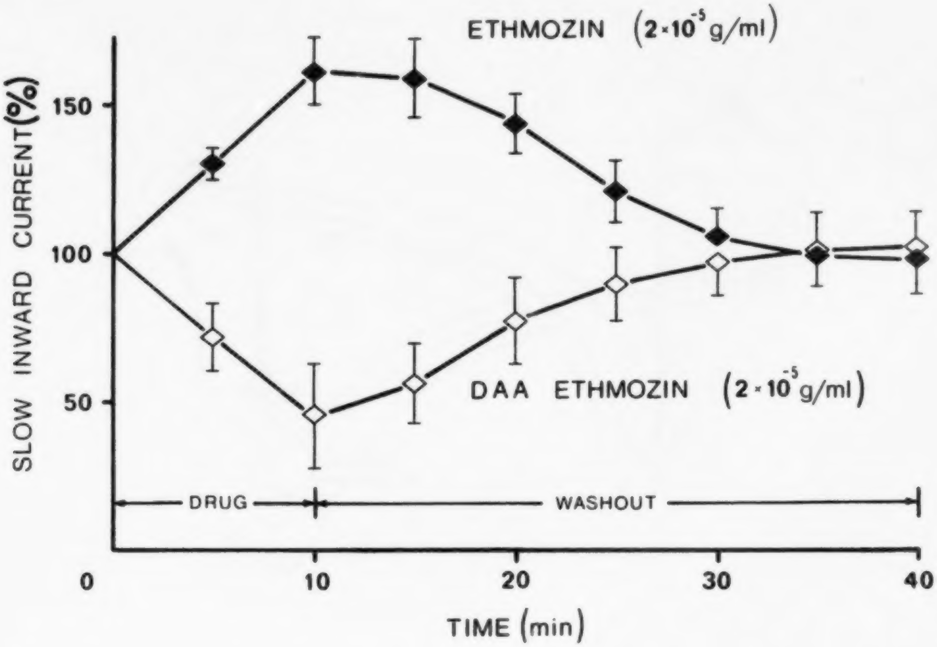

FIGURE 8. Change in the slow inward current of the frog atrial trabecula exposed to ethmozin ($2 \cdot 10^{-5}$ g/ml) and ethmozin DAA ($2 \cdot 10^{-5}$ g/ml), and dynamics of the slow current during the period of washout with Ringer's solution containing no phenothiazines. Graphs show M ± σ obtained in eight experiments. The abscissa shows the values of the slow current expressed in percent of the control.

myocardium was found (14-16). However, in later works it was noted that the effect of lidocaine on the excitability of the myocardium is clearly manifested in hypoxia and with an elevated extracellular concentration of potassium ions (17,18). Furthermore, whereas in the intact myocardium lidocaine does not cause changes in the rate of conduction of excitation, this rate is retarded in an ischemic zone (19). Measurements of the parameters of excitability of the myocardium under clinical conditions have shown that therapeutic doses of lidocaine, at which its antiarrhythmic effect appears, do not lead to significant changes in the rate of conduction of excitation and refractory periods in the myocardium of the atrium and ventricles (20,21). Thus, with lidocaine we see a significantly higher sensitivity of the damaged myocardium in comparison to the intact myocardium to the effect of antiarrhythmic preparations.

In a recent study (6), it was shown that lidocaine blocks the propagation of excitation in myocardial segments isolated from a zone of ischemia; tetrodotoxin had an analogous effect. These data indicate that the effects of lidocaine and of tetrodotoxin on the myocardium after infarction are similar, and agree well with our results, indicating potentiation of the antiarrhythmic effect of lidocaine in the late stage of experimental myocardial infarction by tetrodotoxin. Thus, lidocaine, like ethmozin and mexitil, influences the fast inward sodium current.

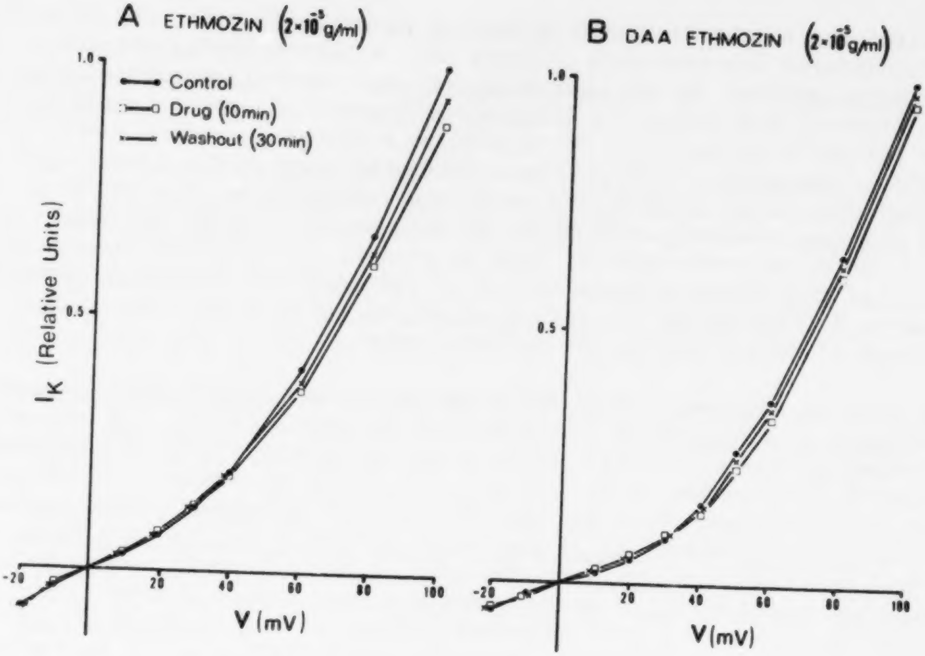

FIGURE 9. Effect of ethmozin currents ($2\cdot10^{-5}$ g/ml) (A) and ethmozin DAA ($2\cdot10^{-5}$ g/ml) (B) on outward currents in the frog atrial trabecula. Graphs A and B show data from one experiment with ethmozin and one experiment with ethmozin DAA. Graphs show stable values of outward current measured 0.5 second after disconnection of each stage of depolarization. Unit of current used was its value obtained with depolarization of the membrane by 100 mV from the resting potential. ●—● = Control. □—□ = 10 minutes phenothiazine. x—x = 30 minutes washing with Ringer's solution containing no phenothiazine. Analogous results were obtained in four other experiments.

The hypothesis that the decrease in sodium current is the reason for relief of arrhythmias is further supported by data from comparing the antiarrhythmic effect of ethmozin and ethmozin DAA with the capability of these phenothiazines to influence the fast inward sodium current. In comparison to ethmozin, ethmozin DAA has a longer antiarrhythmic effect and a stronger influence on the fast inward sodium current.

Our results do not enable us to resolve how the decrease in sodium current leads to an antiarrhythmic effect. One factor might be blockage of the conduction of excitation within the limits of the infarction zone. This possibility was suggested by Lazzara et al. (6).

To answer the question of the mechanism of development of arrhythmias and the causes of antiarrhythmic effects of preparations in the late stage of experimental myocardial infarction, it might be useful to look at the following facts: (a) antiarrhythmic preparations (ethmozin, mexitil, lidocaine, etc.) can stop rhythm disorders 24 hours after occlusion of a coronary artery and can eliminate rhythm disorders caused by aconitin (1,2,4); (b) tetrodotoxin, administered intravenously, blocks both aconitin rhythm disorders (22) and arrhythmias arising in the late stage of experimental myocardial infarction (see figure 1 and table 1); (c) the development of ectopic excitation under the influence of aconitin is related to a change in sodium ion permeability (23). Comparison of these facts indicates that in the late stage of experimental myocardial infarction, among other mechanisms of arrhythmia formation, mechanisms similar to aconitin ectopic excitation may develop. This hypothesis could, in principle, be used to explain the data in our work if one considers that the antiarrhythmic effect of tetrodotoxin, ethmozin, mexitil, and lidocaine is related to the direct effect of these substances on the ectopic focus of excitation in the ischemic zone.

Many studies have emphasized the important role of the nervous system in the development of arrhythmias in myocardial ischemia (24-27). In the early stage of experimental myocardial infarction, during which time rhythm disorders are related to slow conductivity (28-31), the growth of sympathetic tonus, affecting the slow response of the myocardium in the ischemic zone, is facilitated by electrical instability of the heart (25,27). In the late stage of experimental myocardial infarction, the role of the autonomic nerves in the development and maintenance of arrhythmias has been little studied. The data obtained in our work on the antiarrhythmic effect of tetrodotoxin or tetrodotoxin in combination with ethmozin, mexitil, and lidocaine could be explained by changes in the tonus of the autonomic nerves due to blockage of the fast sodium channels in the nerve tissue. Further experiments should resolve the extent to which the effect of antiarrhythmic substances in the late stages of infarction is related to their effect on the autonomic nervous system.

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PART III

PATHOLOGICAL ANATOMY AND NEURAL ACTIVITY

HISTOPATHOLOGY OF THE MYOCARDIUM IN CASES OF SUDDEN DEATH

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ABSTRACT. Histological study of the heart was performed in 105 cases of sudden death, of which 100 cases resulted from coronary heart disease (CHD); in 5 cases the cause of death could not be established. In 98 percent of the cases, the victims were men from 30-60 years of age (with a mean age of 48 years). Among those who died of CHD, 98 percent had stenosing atherosclerosis of one or more coronary arteries, 13 percent had recent myocardial infarction (MI), 38 percent had postinfarction cardiosclerosis, 24 percent had nonuniform filling of the myocardium with blood, provisionally considered a sign of myocardial ischemia, 29 percent showed no macroscopically visible changes in the myocardium, 18 percent had a recent coronary artery thrombosis, 9 percent had thrombosis combined with MI, and 9 percent had myocardial ischemia. In 34 of the 105 cases, succinate dehydrogenase and phosphorylase activity was histochemically determined in the heart tissue in order to study early ischemic myocardial damage. Foci of early ischemic myocardial damage were found in 18 cases; in 9 cases these foci were combined with postinfarction scars, and in 5 cases they were found with recent MI. Microscopic study of the hearts of the CHD victims revealed both old and recent changes characteristic for this disease. The old changes were considered the result of large or small MI sustained or as the result of chronic increasing myocardial ischemia without coronary catastrophe. Three types of recent myocardial changes were distinguished: (a) MI, (b) early ischemic myocardial damage, determined histochemically, and (c) damage to the myocytes revealed by Rego and Masson staining. The last changes were tentatively classified as metabolic changes. The significance of various types of changes in the genesis of sudden death is discussed.

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INTRODUCTION

Sudden cardiac death (SCD) is one of the most important problems in public health today. It is the main cause of sudden death outside the hospital. Several reports have been published on the morphological changes in the myocardium and coronary arteries in SCD (1-6). However, most of these studies were performed using ordinary histological methods, involving autopsies performed long after death for patients who died suddenly outside the hospital. In 1977-1979, combined pathological, histological, histochemical, and biochemical studies were performed at the National Cardiology Research Center on cases of sudden death which occurred outside the hospital. Autopsies were performed not more than 3-6 hours after death.

MATERIALS AND METHODS

This report presents the results of a histological and histochemical study of the myocardium in cases when death occurred at a precisely determined time before witnesses, on the street, at work, in a transport vehicle, hotel, motion picture theater, etc., and in the daytime (not at home and not at night).

The cases studied did not include any victims of sudden death with obvious noncardiac cause, i.e., brain hemorrhage, thromboembolism of the pulmonary artery, epilepsy, etc. Cases of sudden death due to alcoholic cardiopathy and mixed cases (combination of alcoholic cardiopathy and coronary heart disease) were also excluded from the study; these are reported on in a separate report.

Selection and processing of materials were performed using a system previously tested. Specimens were removed from standard sections of the unfixed heart: six sections from the anterior and posterior walls of the left ventricle at three levels (2 cm from the apex, at the middle level, and 2 cm below the fibrous ring of the mitral valve), from the interventricular septum at the same levels (three sections), at the basal and middle levels of the anterior and posterior walls of the right ventricle (four sections), from the anterior and posterior walls of both atria (four sections), and from the area of the sinus and atrioventricular nodes (four sections). A total of 21 specimens were taken. These, in turn, were divided into several components, one of which was fixed in paraffin; another was used for preparing sections on a freezing microtome for lipid staining, and a third was placed in a cryostat for histochemical study. Of the materials taken, 105 cases have now been studied histologically. Microtome sections were stained with hematoxylin-eosin, Masson trichrome stain, Rego stain, toluidine blue, and fatty red.

In 34 cases where sections were taken within 3 hours after death, histochemical studies were performed. The sections of the myocardium taken according to the plan were rapidly frozen with dry ice and cut in a cryostat at -20° C. Histochemical studies were used to reveal the succinate dehydrogenase (SDH) and phosphorylase activity in the sections. These two particular enzymes were not selected by chance. Phosphorylase, as is well known, is extremely sensitive to oxygen shortage. The experimental materials demonstrated that, within 15 minutes after development of a local circulatory disorder in the myocardium, a zone of ischemia clearly begins to appear in which the histochemical reaction to phosphorylase is absent. Phosphorylase is also not found in the area of recent infarctions, and in order to differentiate these later changes from acute ischemic changes, SDH activity was determined using the middle sections. It was shown that the histochemical reaction with nitro-B-tetrazolium begins to change in the myocardium no earlier than 5-6 hours after local disruption of coronary circulation (7). It is not possible to determine precise boundaries of the ischemic zone in the myocardium in this case. Thus, in foci of local ischemia, SDH activity appears macroscopically unchanged and is absent only in the area of the myocardial infarction for a period of at least 5-6 hours.

The activity of the two enzymes is also absent in postinfarction scars and all types of cardiosclerosis. Consequently, comparison of the results obtained by determination of these two enzymes allows us to detect foci of acute ischemic myocardial damage and to differentiate them from older ischemic lesions.

We must emphasize that this type of work requires fresh autopsy material. The longest possible time for determination of phosphorylase is 3 hours after death, and therefore this reaction was performed and used for only 34 of the 105 cases of SCD. Up to this time, the stained end product of the histochemical reaction to phsophorylase begins to diffuse from the myocytes; therefore, these preparations can be evaluated only macroscopically. This evaluation reveals rather precisely zones without stain, which can be considered centers of earlier ischemic myocardial damage.

RESULTS

The materials studied were subdivided into the following two groups:

- Coronary heart disease (100 observations)
- Undetermined cases, where neither heart disease nor any other cause of death could be established (5 cases).

Coronary Heart Disease

The hearts of male sudden death victims from 30 to 60 years of age (with a mean age of 48 years) were studied. The degree of atherosclerosis of the coronary arteries was estimated based on the presence of stenoses in the arterial lumen. Stenoses of more than 50 percent of the lumen were found in 92 percent of the cases; stenoses of less than 50 percent of the lumen were found in 6 percent of the cases. Thus, the total number of cases with stenosis of one or more coronary arteries was 98 percent; 2 percent of the cases showed no stenosis. Usually, multiple stenoses of one or more branches were seen (in 77 percent of the cases with stenosis). Isolated stenoses of one branch of the coronary arteries were found less frequently (in 23 percent).

Primary MI was discovered in 9 percent of the cases, recent changes in 5 percent, and scars in 4 percent. A second recent MI was found in 4 percent of the cases. Thus, the frequency of recent MI in the observations was 13 percent. A combination of MI with thrombosis of a major branch of the coronary artery was found in 9 percent of the cases; in 4 percent, the MI was not combined with thrombosis.

Postinfarction cardiosclerosis was found in 38 percent of the cases; a combination of postinfarction cardiosclerosis and a recent MI was observed in 4 percent of the cases; and only postinfarction scars were seen in 34 percent. Of 34 cases with postinfarction cardiosclerosis, 15 either had old organized thrombi or occlusion of the coronary artery lumen by atherosclerotic plaque.

In 24 percent of the observations, macroscopic study revealed nonuniform filling of the myocardium, which was considered to be a sign of myocardial ischemia. In 29 percent of the cases, autopsy revealed no recent MI, no postinfarction scars, and no ischemic areas, but there was stenosis of the coronary arteries. Fresh coronary arterial thromboses were found in 18 percent of the cases; the thrombi were combined with MI in 9 percent of the cases, and with myocardial ischemia in 9 percent of the cases.

Microscopic study revealed changes, both old and recent, which are characteristic for CHD. The old changes were considered the results of large and small area MI, or as a result of chronically increasing myocardial ischemia without coronary catastrophe.

In postinfarction states, the presence of fibrous scars replacing various amounts of muscle tissue was always noted. The scars usually had a few thin-walled vessels with complete disappearance of the preceding vascular microbed. The muscle fibers in the immediate vicinity of these scars, sometimes seeming to penetrate through the scars, were either atrophied or significantly thickened, with large polymorphic nuclei. The myocardium outside these scars seemed little changed in some segments, while in other segments of the same heart we always found nonuniform hypertrophy of individual bundles or layers of muscle fibers, always combined with various indications of chronic myocardial ischemia.

These signs of chronic ischemia in some cases were seen without postinfarction changes or with only slight postinfarction scars; in other cases they clearly predominated over the scars in volume and extent. Although the changes are quite varied in their morphology, they are distinguished by the fact that, even with the most severe degrees of damage, the myocytes as a structure do not completely disappear and the microvascular bed always remains intact and filled with blood. There is no regularity in terms of the location of these changes. They are either diffuse or in the form of scattered foci over a large area of the myocardium of both ventricles and the septum, but they sometimes merge into larger foci. Microscopically, these changes are characterized by atrophic, dystrophic, and necrobiotic changes of the myocytes with varying degrees of severity and accompanying diffuse or focal interstitial sclerosis.

Least frequently of all, we saw individual scattered foci of several atrophied, thinned muscle fibers, frequently with individual vacuoles in the cytoplasm or nuclei. When there is a quantitative increase in these changes, fields appear, and, in longitudinal sections, layers of greatly thinned, vacuolized, seemingly elongated and stretched out muscle fibers are found, in some of which we see moderately expressed fatty dystrophy of the cytoplasm (figure 1). The bundles or plates of these fibers are frequently wavy, seeming to

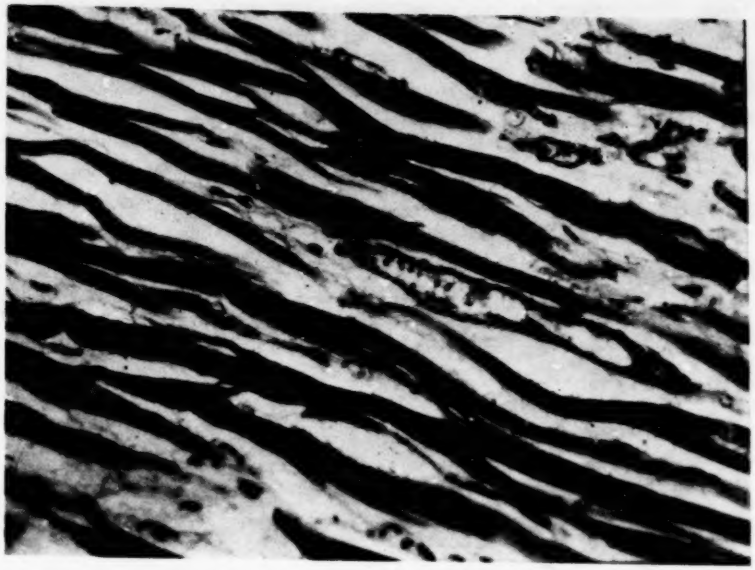

FIGURE 1. A layer of acutely atrophied myocytes. Reticular interstitial sclerosis. Hematoxylin-eosin stain. Magnification x 265.

hang. Between bundles of such fibers we often observed reticular interstitial and perivascular sclerosis.

In addition to this, in the same cases we observed more severe changes of a dystrophic and necrobiotic nature, usually focal, and most frequently localized near the endocardium, which is characterized by progressive disappearance of the sarcoplasm and sometimes the nuclei, but with relative conservation of the sarcolemma of the atrophied myocytes. In the final stage, these sections have the appearance of hollow honeycomb structures, with interlayered interstitial reticular and, less frequently, coarser fibrous sclerosis (figure 2).

Recent changes to the myocardium, which have a more direct relationship to sudden death, were divided into three types.

1. Myocardial infarct, 12-24 hours old, before scarring. These infarcts were all combined with more or less clearly expressed changes in the myocardium, which are characteristic for chronic coronary insufficiency, as noted earlier. In 9 of the 13 cases with MI, thrombosis of one of the branches of the major coronary artery was also found, occurring at the same time and in the corresponding area. The infarcts were in different locations and even in cases with thrombosis were relatively small; in no cases were extensive or transmural infarcts seen.

2. Early ischemic (hypoxic) changes. These changes were determined histochemically. Macroscopically, we noted nonuniform blood content in the corresponding sections of the myocardium. Foci of early ischemic changes in the myocardium were detected histochemically in 18 of 34 cases.

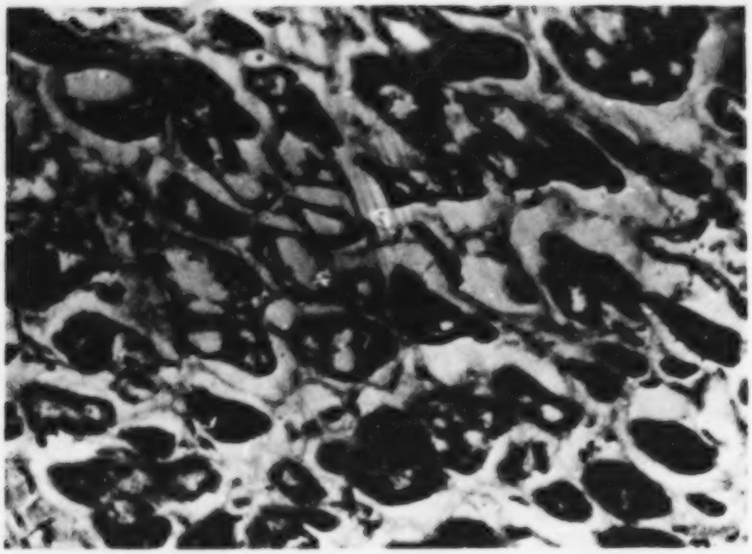

FIGURE 2. Atrophy of myocytes with disappearance of sarcoplasm. Hematoxylin-eosin stain. Magnification x 265.

Of these 18 cases, in 5 there had been an acute MI, and in 3 cases the foci of ischemic damage to the myocardium were individual and corresponded to the infarct area, as determined by the phosphorylase and SDH reaction. In two observations, the main focus of ischemia (infarct) was combined with multiple small foci of acute ischemia in other segments of the heart, found in the form of unstained sections in the phosphorylase reaction alone.

In 4 of the 18 cases, the only changes found in the myocardium were acute ischemic lesions, revealed by the phosphorylase reaction. One example of this group of cases is the heart of the deceased patient G., 50 years of age, in which stenosis was found in the initial segment of the anterior descending branch of the left coronary artery, constricting the lumen of the artery by 90 percent. In the left marginal branch and right coronary artery, plaque was found, constricting the lumen of the vessels by 50 percent. No focal changes were found in the myocardium upon autopsy. In histochemical study of the myocardium with phosphorylase reaction, foci of acute ischemia were found in the posterior wall of the left ventricle and interventricular septum, which were not revealed by the SDH reaction. In all remaining segments of the myocardium, uniform, intensive reactions to phosphorylase and SDH were found.

In 9 of 13 cases, foci of ischemia in the myocardium were combined with postinfarction scars (in 4 cases) and small focal cardiosclerosis (in 5 cases). In the myocardium of these victims, both the phosphorylase and SDH studied indicated no reaction in areas of postinfarction cardiosclerosis and cicatric changes of the cardiac muscle, with the exception of cell elements having respiratory enzyme activity. In addition to these changes, foci of acute ischemia were found, in which SDH activity appears unchanged, but phosphorylase disappears. An example of this group of cases is the heart of patient K., 44 years old, in whom we found clear postinfarction cardiosclerosis in the anterior wall of the left ventricle and the interventricular septum, together with a recent ischemic focus in the posterior wall of the left ventricle, and postinfarction scars in sections not stained in reaction to phosphorylase or SDH. The recent ischemic focus was determined by the lack of phosphorylase reaction, whereas the SDH reaction appeared normal (figure 3).

In the myocardium of sudden death victims where there were foci of acute ischemic damage without histologically diagnosed MI, four cases showed individual lesions, which occupied large areas of the myocardium and traced to various levels of the corresponding segment of the myocardial wall. In nine cases, the foci were multiple (e.g., two to three foci) and could be found both in one segment of the myocardium, but only in the anterior wall of the left ventricle, or in various sections (e.g., in the left and right ventricles simultaneously, in both ventricles and the interventricular septum, or in other combinations) (figure 4).

The dimensions of these ischemic foci also varied, and a large focus might be combined with smaller ones in other segments. Sometimes we observed in all segments of the myocardium only small foci 2-3 mm in diameter or medium-sized foci up to 5-6 mm in diameter.

A large group of persons (16 cases) died of sudden death due to acute cardiac insufficiency, with stenotic atherosclerosis of the coronary arteries but with no foci of acute ischemia or recent infarcts in the myocardium. When

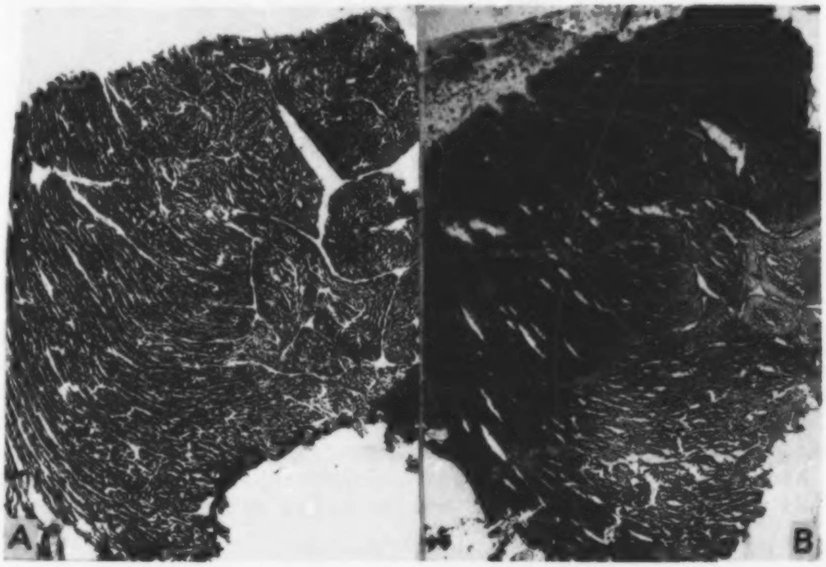

FIGURE 3. Posterior wall of the myocardium of the left ventricle. A: Reaction to succinate dehydrogenase; no visible changes in the enzyme. B: Reaction to phosphorylase; foci of ischemia with no enzymatic activity. Magnification x 30.

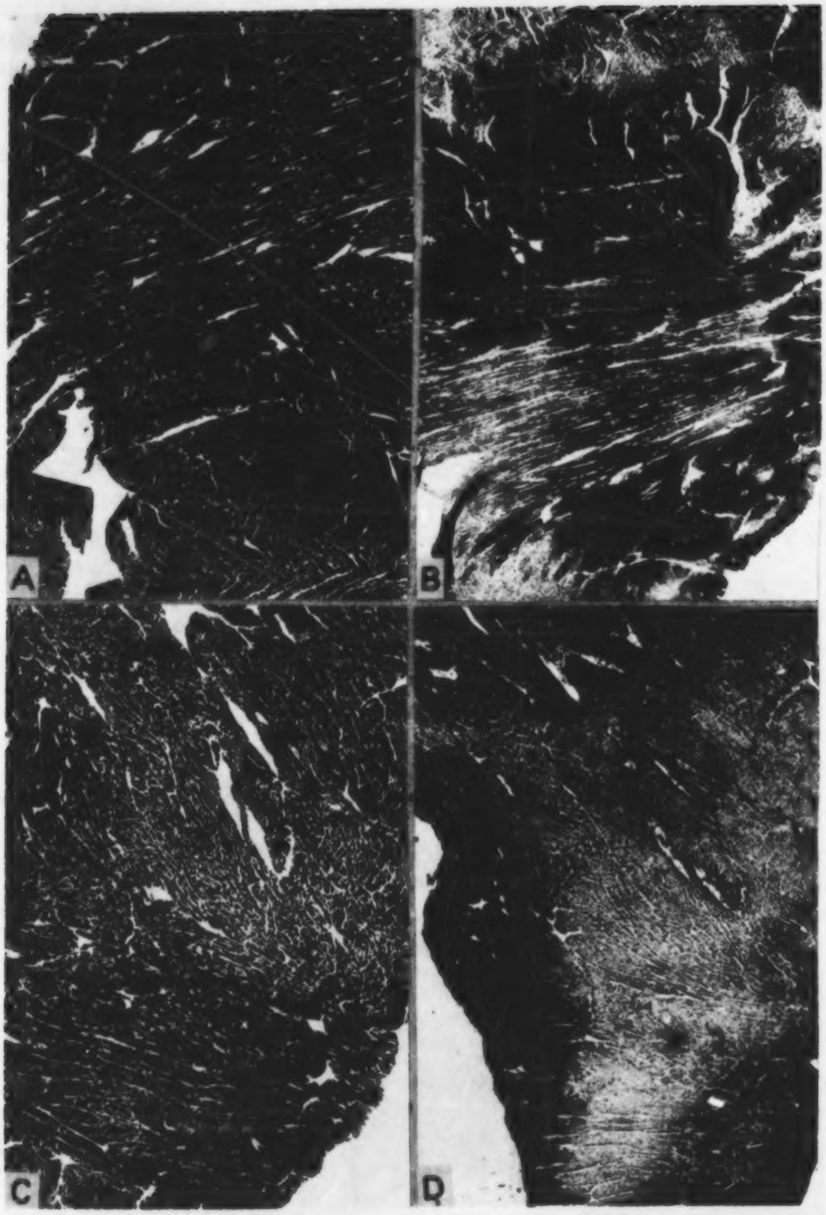

FIGURE 4. Foci of acute myocardial ischemia in various sections. A and B: Anterior wall of the left ventricle. C and D: Interventricular septum. A and C show no changes in the succinate dehydrogenase test. B and D show foci without stain in the phosphorylase test. Magnification x 30.

the activity of both enzymes (SDH and phosphorylase) was measured in the myocardium of these victims, only recent focal changes--scars and areas of cardiosclerosis--were seen in the unstained sections. Our attention was drawn by the fact that in persons who had had cardiosclerosis, the size of the sections without stain in the phosphorylase test was in almost all cases somewhat greater than in the SDH test, although the topography and contours of these zones corresponded completely. This is apparently explained by the fact that the muscle fibers located around the scars and areas of cardiosclerosis suffer from oxygen deficit. Phosphorylase, which is sensitive to a shortage of oxygen, is not found in these myocytes, so that the zones without this enzyme are somewhat larger than those without SDH.

Thus, of 34 cases studied, foci of acute ischemic damage in the myocardium were found in 18 persons, 15 of whom had stenotic atherosclerosis of the coronary arteries.

We must note that localization of ischemic foci in the myocardium does not fully correspond with the severity of stenosis of the artery feeding the segments of the cardiac muscle where a focus of ischemic damage was found. Thus, in cases with severe stenosing atherosclerosis in any of the coronary arteries, when the lumen of the vessel is reduced by 80 percent or more, we frequently found, in addition to the focus of ischemia in the area of the sharply stenotic coronary artery, foci in the area of the myocardium which had coronary vessels with a lower degree of stenosis. An example of such a situation is the heart of patient K., 49 years old, with a left dominant type of circulation. Foci of acute ischemic damage were found in the anterior and posterior walls of the left ventricle, with severe stenosis constricting the lumen of the vessel by 85-90 percent observed only in the left anterior descending branch.

The same can be said of cases with recent coronary artery thrombosis. In most cases, foci of acute ischemia were localized not only in sections of the myocardium with the thrombosed coronary artery, but also in locations supplied by unthrombosed vessels. For example, in patient S., 51 years of age, with a left dominant type heart circulation, a thrombus was found in the left marginal branch, while foci of ischemia were found not only in the posterior wall of the left ventricle, but also in its anterior wall, as well as in the anterior and posterior walls of the right ventricle.

And, finally, foci of acute ischemia were observed in cases with very slight stenosis (four cases). One example is the heart of patient T., 56 years of age, with a balanced type circulation and with plaque in all three coronary arteries, reducing the lumen by only 25-50 percent. However, scars from MI were found, as well as an infarct approximately 2 days old in the posterior papillary muscle of the left ventricle, together with foci of acute ischemia in the anterior and posterior walls of the left ventricle and in the interventricular septum.

3. Lesions of the muscle fibers. Lesions were found upon staining of histological preparations with iron hematoxylin using the Rego method. In serial sections, these lesions corresponded precisely in location with equally clear and distinct changes in the myocytes detected by Masson trichrome staining,

and also in the periodic acid-Schiff (PAS) reaction, and much less constantly but still quite clearly in some cases with the hematoxylin-eosin stain; they did not correspond either topographically or in terms of characteristic features with early ischemic or hypoxic changes determined histochemically.

Rego's stain reveals lesions as intensively stained black or dark gray muscle fiber (figure 5) (together or separated in several sarcomeres) (figure 6), with a sharp boundary between damaged and undamaged sections, which are pale gray in color. The number and distribution of lesions vary widely in different sections of the myocardium. Most frequently, they are seen in the left ventricle and interventricular septum, less frequently in the wall of the right ventricle, and still less frequently in the atria, although in some cases the relationship was the reverse. The lesions might be small focal and less frequently scattered or multiple and densely located over significant areas; they might be localized primarily beneath the endocardium, epicardium, or in the mass of the myocardium. At times, they encompassed atrophied or hypertrophied fibers (figure 7), although by no means always or everywhere. Frequently, they developed in fibers of normal volume.

In serial sections treated with Masson staining, these lesions can be isolated by the pronounced lightening of the basic tone of staining of the sarcoplasm (figure 8). In place of the saturated dark red color, the damaged myocytes or fractions of myocytes are pale pink in color, and with this coloration (in contrast to Rego's method in which, due to the intensity of the tones, intracellular structures are quite difficult to distinguish), slight changes in the nuclei of the myocytes are easy to see, but there are usually no intraventricular structures, including longitudinal and transverse structures

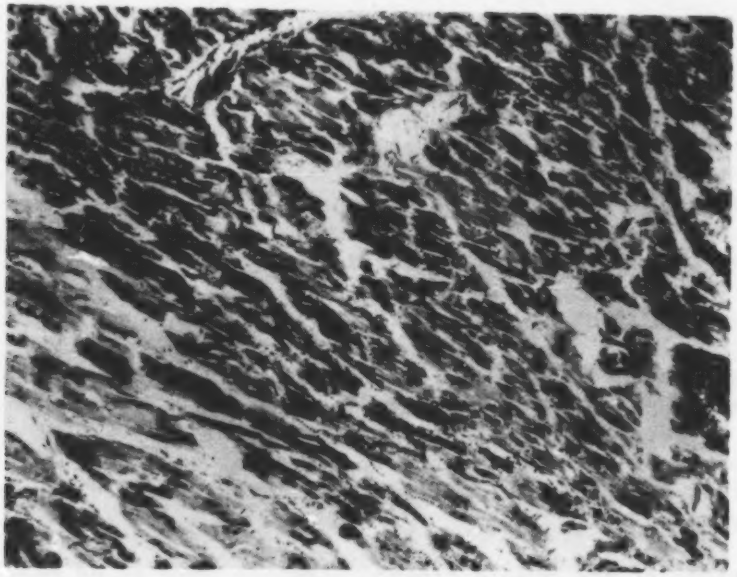

FIGURE 5. Small, partially merged lesions of myocytes with Rego staining. Magnification x 107.

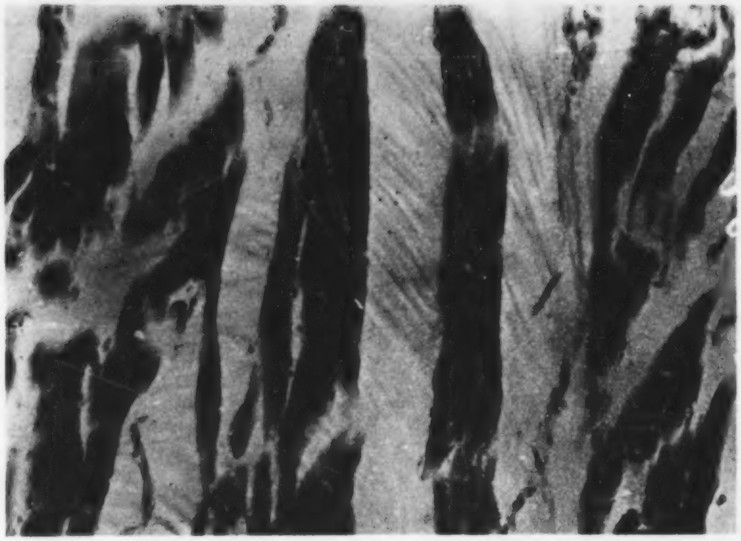

FIGURE 6. Focal damage of individual muscle fibers or parts of fibers revealed with Rego staining. Magnification x 265.

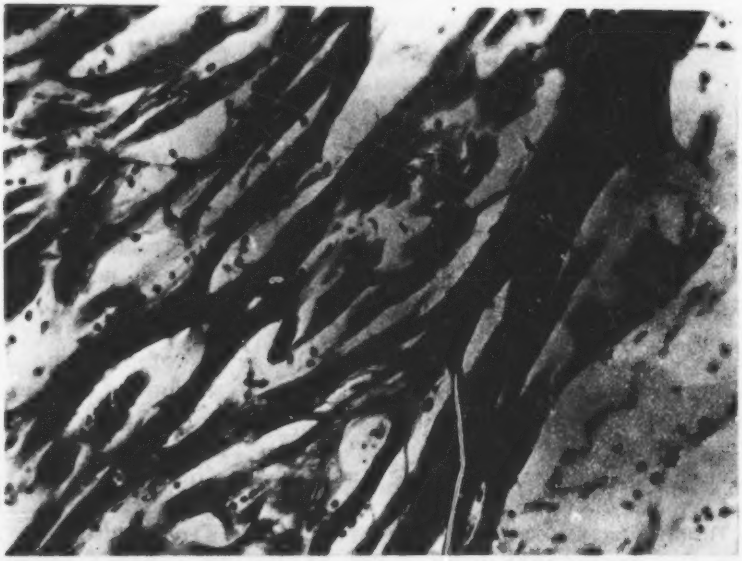

FIGURE 7. Lesions in hypertrophic and atrophic muscle fibers with Rego staining. Magnification x 265.

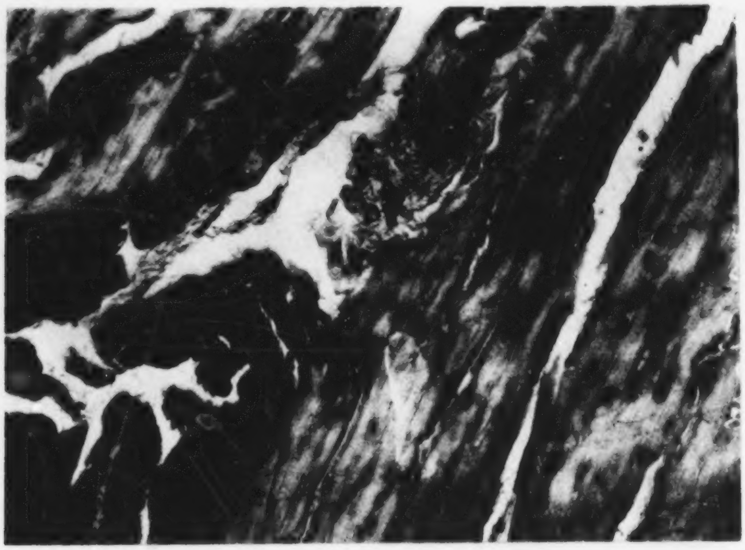

FIGURE 8. Clarification of cytoplasm of damaged myocytes with Masson staining. Magnification x 265.

(sometimes in individual fibers they are very weakly expressed in places). With this same stain, we see sarcolemmal changes in the lesions much more clearly than with Rego's stain; the sarcolemmas become uneven and swollen, sometimes losing their continuity over short sections; the muscle fibers themselves are sometimes deformed and become ampule-shaped (figure 9).

With the PAS reaction, the same lesions are stained noticeably more intensely. They can be observed much less constantly and clearly with the hematoxylin-eosin stain. The intensity and color of the sarcoplasm remain the same, but it appears homogeneous as if varnished.

The reaction to sulfhydryl groups and the Kuns immunofluorescent method to plasma proteins, according to preliminary data, were negative in the areas of lesions.

The small vessels and capillaries in the areas of lesions are most frequently completely unaltered; sometimes they are expanded and polyemic, but with no signs of circulatory disorder.

In some cases, the lesions merge into large foci or extend over a considerable portion of one of the walls of the left ventricle or interventricular septum. These deserve particular attention, since they are also observed in MI cases in the noninfarct zone. The following case is instructive in this respect.

Patient V., 49 years of age, died suddenly at work. His heart size was 450 g, and we found significant atherosclerosis of the coronary vessels with stenosis of the lumen up to 75 percent and an obturating thrombus with signs

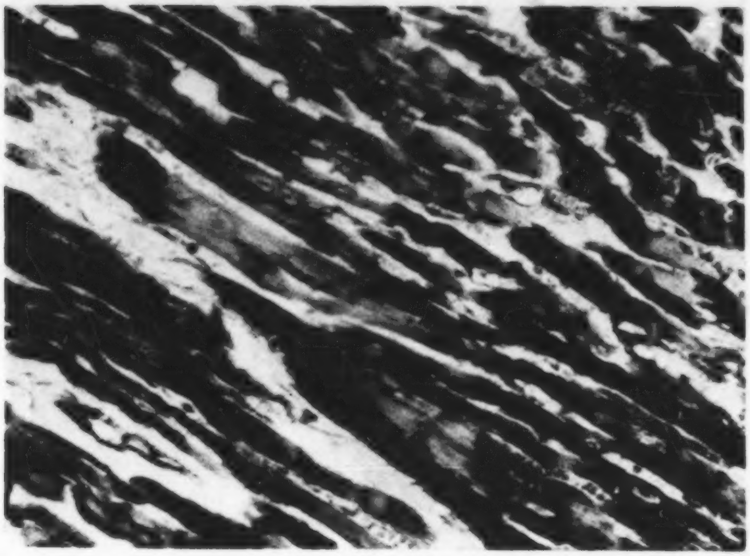

FIGURE 9. Deformed muscle fibers with Masson staining. Magnification x 265.

of organization in the right coronary artery 7 cm from its origin. In the anterior wall of the right ventricle in its middle third section, we observed a rather broad, almost transmural MI, represented by coagulation necrosis of muscle fibers surrounded with a broad zone of granulation tissue with a large quantity of cell elements. Many muscle fibers in the immediate vicinity of the infarct had multiple merged lesions, whereas the necrotized fibers in the zone of the infarct were stained as undamaged fibers, with the exception of individual atrophied fibers, which stained dark black in Rego's stain. In noninfarct segments of the right ventricle, there were scattered small focal lesions and, in places, moderate-sized merged lesions with no ordered localization (figure 10). The same was also noted in the interventricular septum and in the upper segments of the anterior and posterior walls of the left ventricle.

In the middle third of both walls of the left ventricle and in the posterior lower (apical) third, the picture was quite different. Here we saw a complex, massive merged lesion involving most, but not all, of the muscle fibers extending in transverse cross-section over the entire middle portion of the wall, with comparatively narrow subepicardial and subendocardial strips remaining undamaged. In preparations treated with Masson staining, the undamaged area appeared transparent, while with hematoxylin-eosin staining, the coloration was nonuniform and usually considered to be a result of processing defects.

It must be noted that in several cases of SCD with CHD with stenosing atherosclerosis, these acute lesions of the myocytes were absent or were minimal, appearing only as sparsely scattered small foci.

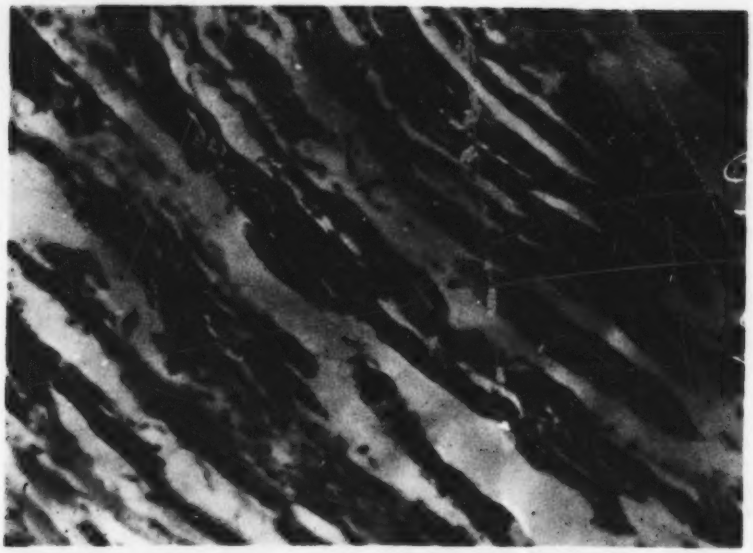

FIGURE 10. Damage to myocytes in noninfarction sections of the myocardium of the left ventricle. Rego staining. Magnification x 265.

Undetermined Cases

This group included five cases which were divided into two subgroups: (a) those in which autopsy revealed neither cardiac pathology nor any other disease and the cause of death was not established (three cases), and (b) those in which there was no disease of the heart or other organs and the cause of death was unclear (two cases), but before death, acute respiratory disease in the pulmonary form had been diagnosed in one case and angina had been diagnosed in the other. The three cases in the first subgroup are described below.

The first case is a female university student, 19 years old, who died on the street near the building where she lived. Witnesses reported that she had been walking at an even, strolling pace, and then suddenly stopped and slowly dropped to the sidewalk. An autopsy was performed 30 minutes after death. The only unusual feature was significant oligemia of the lungs and absence of blood or clots in the right ventricle. Questioning of relatives and examination of her medical history showed that in childhood heart murmurs had been heard and at one time a congenital heart defect had been suspected. She had lagged in growth and physical development for a long time and had seen an endocrinologist and other specialists for this reason, but during the 2 years previous she had become physically well developed and was a strong woman, with no complaints, and involved in sports.

The second case is a sportsman, a rated master of sports, a mountain skier, 24 years old, who died in a barber shop seated in the chair. On autopsy, no signs of pathology were seen, and nothing significant was found in his medical history.

The third case is a ballerina, 29 years old, who died on the ice after a few minutes of skating. Autopsy revealed no pathology, but relatives reported that a year previous, during a medical abortion, she had experienced clinical death, was revived with difficulty, and that the reason had not been established.

In all three cases, chemical studies were performed on the organs and blood; bacteriologic and virologic testing was also conducted, but all tests were negative. Nothing significant was found, histologically or histochemically. Rego and Masson stains revealed minimal foci of damage in the form of individual, very small, and sparsely scattered foci in the sections taken from the wall of the left ventricle.

The two cases of the second subgroup are described below. A young man, 17 years old and a student, complained of chest pains for several days, and was put on bed rest in the dormitory with a temperature of about 38° C. An hour before death, several friends came to visit and they began roughhousing; the victim then suddenly died.

The second case is a worker from Uzbekistan, 40 years old, on temporary duty in Moscow, who died suddenly during the day in the hotel. For 2 days previous he had felt weak and had a subfebrile temperature. He went to see the hotel doctor, who diagnosed acute respiratory disease, but he did not go to bed and continued to visit various institutions.

In both cases, clinical diagnosis was confirmed upon autopsy (lacunar angina and inflammation of the respiratory tract), but the cause of death remained unclear.

Upon histological examination, only Rego and Masson stains revealed in both cases many widespread places of massive damage to the myocytes in both ventricles and the interventricular septum.

The Conductive System of the Heart in Cases of Sudden Death

The conductive system of the heart was studied by a modification of the Lev method. Histological study of the nodes and pathways of the conductive system of the heart was performed in 100 cases, and enzymatic and histological study was conducted in 34 cases. The activity of the following enzymes was determined: succinate dehydrogenase, phosphorylase, acetylcholinesterase, acid phosphatase, and monoaminooxidase.

Upon microscopic examination of the sinoatrial node, the following chronic pathological changes were found: (a) stenosis of the sinus artery (in 4 cases); (b) a significant increase in the proportion of connective tissue in the node (fibrosis or fibroelastosis of the node) with a simultaneous decrease in the number of specialized muscle fibers (in 20 cases); (c) lymphoid infiltration of the tissue of the node (in 12 cases); and (d) changes in the nerve ganglia located near the node, including hyperchromatosis, vacuolization, eccentric displacement of nuclei, pyknosis, and cardiolysis (in 12 cases).

Acute dyscirculatory and necrobiotic changes were much less frequently seen. These included (a) thrombosis of the sinus artery; (b) myocytolysis of individual specialized fibers, eosinophilia, homogenization, and swelling of the sarcoplasm of the myocytes (in two cases); and (c) microscopic hemorrhages in the vicinity of the node, partially penetrating into the tissue of the node and of the surrounding nerve trunks and ganglia (in eight cases). These changes might be found in various combinations in one victim.

Still rarer were pathological changes in the lower segments of the conductive system of the heart, including (a) interruption, due to a congenital developmental anomaly, of the conductive pathways at the point where the atrioventricular node is connected to the body of the His bundle (in one case); (b) sclerotic changes in the body of the His bundle and proximal branches of the limbs of the His bundle (in two cases); and (c) presence in the fibrous body of sections with tissue resembling in its structure the tissue of the atrioventricular node (in one case).

The activity of the enzymes mentioned above was generally lower in the conductive system than in the contractile myocardium. A sharp decrease in phosphorylase activity with high activity of the other enzymes in the tissue of the sinus node was found in only two cases with a recent obturating thrombosis of the initial segment of the right coronary artery. These changes indicate early ischemia of the tissue of the sinus node. In the remaining cases, even with severe stenosing coronary artery atherosclerosis, no signs were found which indicated hypoxia of the sinus and atrioventricular nodes. This indicates high stability of the conductive system of the heart to the development of oxygen deprivation.

The data show that acute pathological changes in the conductive system of the heart are seen in only a small percentage of sudden death cases. Chronic pathological changes are found with a frequency analogous to that seen in CHD patients who did not die suddenly (8-10); therefore, their pathogenetic significance in sudden death is doubtful.

The data we have presented agree with assumptions made earlier (11,12) about the relative role of the conductive system of the heart in the genesis of lethal arrhythmias and sudden death.

DISCUSSION AND CONCLUSIONS

At the present stage of our work, we can provide only some very preliminary results and conclusions. During the course of the study, we found two basic categories of states in which sudden death occurs: (a) in cases in which the main cause of death is more or less obvious and (b) in cases in which the main cause of death is doubtful, i.e., cannot be confirmed, but at the same time no strongly manifested extracardiac causes are evident.

Sudden cardiac death is related by most authors to ventricular bradycardia, which may result from electric instability of the myocardium with various preceding cardiac rhythm disorders, acute myocardial infarction, and primary ventricular fibrillation not related to focal changes in the myocardium. As has been shown by observing persons brought back from sudden death, only one-fourth of them had or subsequently suffered acute transmural MI. In approximately one-half of these cases, there were signs of focal necrosis in the myocardium, while the others were apparently cases of primary ventricular fibrillation not related to acute focal myocardial ischemia and subsequent necrotic changes (13-19).

The data obtained in the present study show that in approximately 60 percent of the cases of sudden death, foci of acute ischemic damage were found histologically in the myocardium, which could lead to ventricular fibrillation. In most of these cases, the ischemic areas were localized in the coronary artery bed with severe atherosclerosis or fresh thrombosis. However, in a number of cases, areas of acute ischemia were also found in the coronary artery bed with only slight atherosclerotic changes. Obviously, coronary artery spasm could be important in the pathogenesis of these ischemic changes; spasm of this type has been repeatedly documented with increasing use of coronarography (20-25).

Our data also indicate that, in 40 percent of the cases, no acute ischemic changes in the myocardium were found, although the degree of coronary atherosclerosis in these patients was the same as in the group of patients who died with ischemic foci. Two explanations are suggested for this finding: (a) the duration of acute ischemia from beginning to moment of death was so brief (less than 10-15 minutes) that no changes in phosphorylase activity in the myocardium which could be detected histochemically had time to occur, and (b) death resulted from ventricular fibrillation unrelated to myocardial ischemia. This second suggestion agrees with the report of Weaver et al. (19), who found no ischemic changes in the myocardium of persons who had died suddenly on the street, were revived, and then taken to the hospital, i.e., persons included in the group with primary ventricular fibrillation.

We can also assume that the histochemical reactions to phosphorylase and SDH reveal not only the actual ischemic damage and perhaps not so much as they reveal tissue hypoxia of the myocytes which is not always acute in nature. Lesions of another type, revealed by Rego and Masson stains, were seen much more frequently and in most cases were expressed quite strongly. The essence of these lesions and the mechanism of their development remain unclear. They usually do not correspond in localization with the ischemic damage, and the nuclei change little, although the sarcoplasm is significantly damaged. For the time being, we arbitrarily and tentatively consider these to be metabolic lesions. If this is confirmed in the future, the combination of these changes in the heart in most cases of sudden death from CHD will create the impression of a metabolic catastrophe. It is worth noting that this is what we observed in the intact segments of the heart in cases of myocardial infarction.

In the few cases where both ischemic and metabolic lesions are absent, but there are slight signs of chronic coronary insufficiency, the cause of death remains essentially unclear, and, in spite of the formal diagnosis of coronary (or ischemic) heart disease at autopsy, these cases should be classified more properly as unknown cause of death.

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NEURAL LESIONS IN THE HEART AND SUDDEN DEATH

Thomas N. James

ABSTRACT. Electrical instability of the heart is a major factor leading to sudden unexpected death. Neural control permits many rapid adjustments which act to preserve stability of the heart, but little is known of diseases which may affect the intracardiac nerves. From a continuing clinicopathological correlative study of sudden unexpected deaths it has become apparent that nerves and ganglia of the heart are often abnormal, particularly in the vicinity of the conduction system. Furthermore, focal disease occurs in ventricular nerves (e.g., in long QT syndromes) and may derange repolarization, thus favoring electrical instability. Cardioneuropathy can be secondary to heritable disorders (e.g., Friedreich's ataxia), or generalized infections (e.g., diphtheria), or systemic diseases (e.g., polyarteritis nodosa). Primary cardioneuropathies include especially viral ganglionitis or neuritis. Any cardioneuropathy may alter tonic or reflex neural control of cardiac rhythm, conduction, or repolarization. Response to humoral factors such as cardioactive ions or pharmacological substances can also be deranged. For example, some aliphatic aldehydes prolong repolarization while others act like tyramine in releasing norepinephrine from cardiac stores. One of the latter group (acetaldehyde) is the principal metabolite of ethanol. Appropriate examples are presented for all of these anatomical changes and physiological experiments. To unravel the pathogenesis of sudden unexpected death, a better understanding of the normal and abnormal anatomy and function of the nerves of the heart will be useful.

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INTRODUCTION

Sudden unexpected death may be due to a number of causes which can be readily detected at postmortem examination, such as ruptured aneurysm or pulmonary embolism, but when none of these familiar causes can be found, most investigators now believe that a lethal cardiac arrhythmia is the probable cause of death. It is perhaps less surprising that the heart should sometimes develop some form of fatal electrical instability than the fact that it can remain stable so long and under so many different challenges to its performance. One of the most important stabilizing influences is mediated by the nerves of the heart, which permit it to adjust very rapidly in many ways. For example, circumstances which require either a slower or quicker heart beat nearly always evoke an immediate change in the activity of the vagus or sympathetic nerves, respectively. Everyone now knows that the transplanted heart can function successfully for a long time, and it has necessarily been detached from all extracardiac neural control. However, except for those patients with transplanted hearts, all of the rest of us have nerves which powerfully influence almost every facet of our cardiac performance. We need to know as much as possible about these neural stabilizing influences and thereby to understand better how instability can occur.

As the basis for the following discussion on diseases of the nerves within the heart, two types of personal studies are reviewed. In the first category the emphasis is upon clinicopathological correlations in examples of sudden unexpected death. These correlations include examination of the cardiac conduction system (including its blood supply) and the nerves of the heart. The second category deals with experimental physiological and pharmacological research utilizing particularly the selective perfusion of the sinus node (1,2) or the atrioventricular (AV) node and His bundle (2,3) through their cannulated nutrient arteries in the dog. In this second category the emphasis is upon direct and indirect influences on neural responsiveness by the cardiac pacemaker and conduction system. Presentation of the experimental studies on the dog is given in overview fashion, primarily to illustrate the probable functional significance of some of the abnormal anatomical changes observed within the nerves or ganglia of the human heart.

STRUCTURAL ABNORMALITIES OF THE NERVES AND GANGLIA OF THE HEART

Considering the great importance of cardiac innervation for the proper normal performance of the heart and adaptive changes during all forms of stress, much less attention (in the United States) has been directed at postmortem examination of intracardiac nerves and ganglia than seems merited, especially in cases of sudden death. In this symposium it is fair to acknowledge that our Soviet colleagues have not neglected this subject (4-6). Ironically, of the recent advances in cardiac pharmacology, some of the most dramatically successful have been through the use of drugs which act principally or exclusively by altering neural responsiveness, e.g., the adrenergic beta-receptor blocking compounds. Furthermore, there is growing recognition that most cardioactive drugs have their effects in either greater or lesser measure by their influence on autonomic neural responses, although some of these neuropharmacological actions may be central (e.g., within the brain) rather than within the heart.

Whatever the explanation may be for our comparative lack of interest in neuropathology of the heart, there is mounting evidence that this area may represent a serious oversight. In a variety of examples of sudden unexpected death, following which careful routine necropsy examination yielded no explanation, there have been distinct and impressive abnormalities of the nerves and ganglia within the heart (7-10). While the exact functional significance of these anatomical abnormalities remains to be elucidated, it seems prudent to examine some of the more plausible possibilities, not only to improve our understanding of the terminal events of sudden death, but also to learn how disorganization of neural control can play a role in other more transient disturbances in cardiac function.

Before proceeding with discussion of structural abnormalities, two points of normal anatomy must be considered. One of these is a special intracardiac chemoreceptor present in both human and canine hearts, and the other is the electron microscopic anatomy of neuromuscular junctions in the heart. The distribution of adrenergic and cholinergic nerves within the heart has been discussed previously at the first US-USSR symposium on sudden death (4). And, some of my own experience in this anatomical subject has also been published (11,12).

Concerning the chemoreceptor, there are several important possibilities in the pathogenesis of sudden death. In both human and canine hearts this chemoreceptor receives its primary blood supply from the very proximal portion of the left coronary artery (13). Its histological appearance resembles carotid body (figure 1), and this is true for both human and canine hearts (13). However, its function differs significantly from carotid body in being maximally responsive to 5-hydroxytryptamine (serotonin), in concentrations expected to be present in the vicinity of aggregating platelets. The afferent pathway of a reflex originating within this chemoreceptor is in the thoracic vagal branches (14,15). When maximally stimulated in the dog, this chemoreflex causes virtual doubling of aortic pressure within about 5 seconds (figure 2). Concomitant with a strong sympathetic efferent discharge, the cardiogenic hypertensive chemoreflex also causes a very brief but powerful vagal discharge (13,14,16). This mixture of vagal and sympathetic influences includes the conduction system, and a variety of electrophysiological disturbances are a characteristic feature of the reflex (13). Both from the magnitude and speed of the pressor response and the concomitant electrophysiological disturbances, one can anticipate that participation by the cardiogenic hypertensive chemoreflex may be important in certain examples of sudden unexpected death, particularly in association with left coronary disease (17).

In contrast to the extensive knowledge available about neuromuscular junctions in skeletal muscle, there is very little information about connections between nerves and myocytes in the heart. This is despite the fact that autonomic nerves and ganglia are so abundant in many locations within the heart, including particularly components of the cardiac conduction system (11,12,18-22). In recent electron microscopic studies of the internodal pathways within the heart (23), Sherf and I found a remarkable example of direct contact between a nerve and at least one myocyte within the posterior internodal pathway (figure 3). From multiple thin sections we found that the course of the nerve and the long axis of the myocyte were perpendicular, and that numerous small but nondescript structures in the vicinity were actually a part of the special junction. From

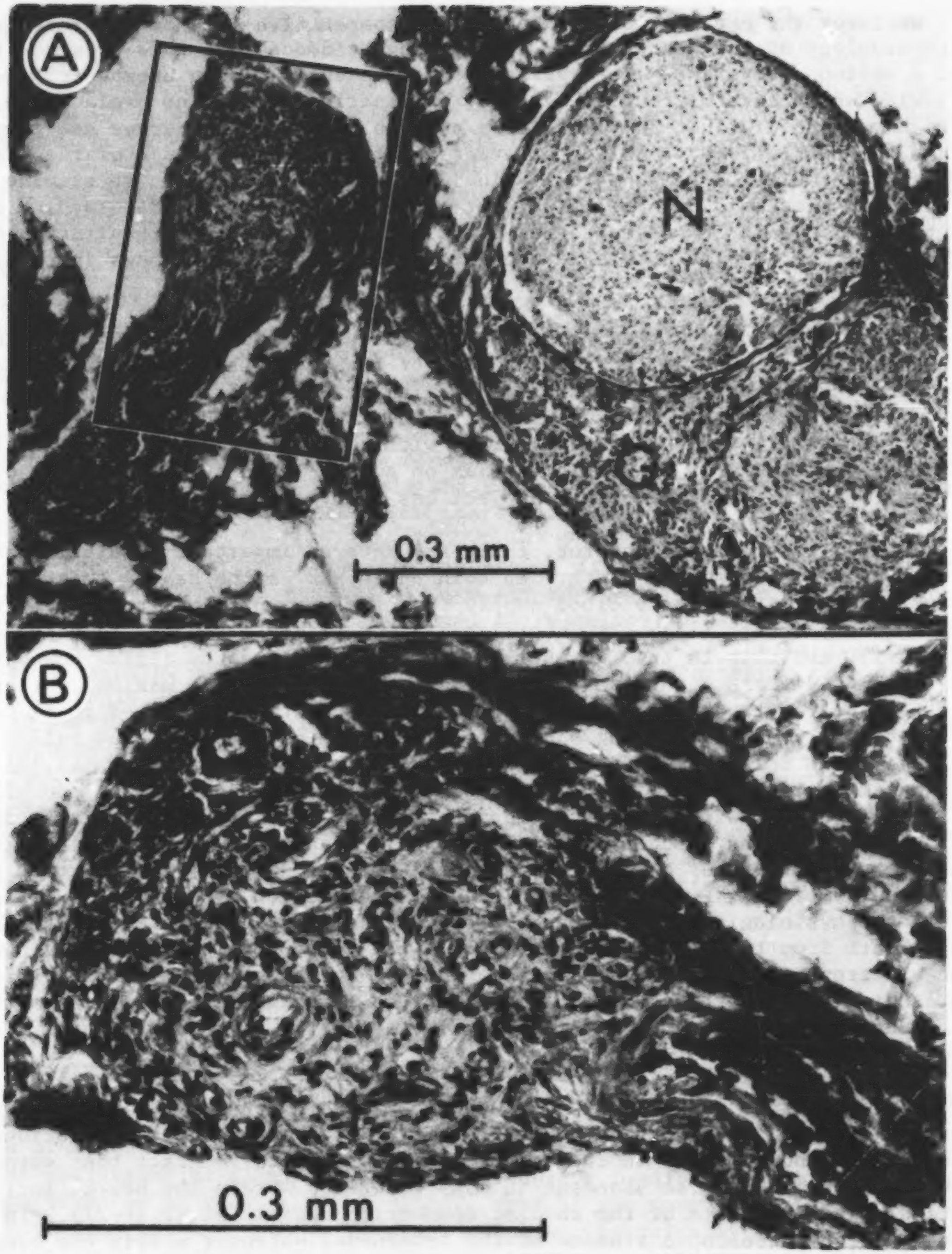

FIGURE 1. The human coronary chemoreceptor is boxed in A and shown at higher magnification in B. N and G mark a nerve and a ganglion near the chemoreceptor. The nutrient artery about which the chemoreceptor is organized originated from the main left coronary artery of this human heart. Magnifications are indicated with reference bars. Goldner trichrome stain was utilized here and in all subsequent photomicrographs except as indicated. [Adapted by permission of the American Heart Association, Inc., from James TN, Isobe JH, Urthaler F: Analysis of components in a hypertensive cardiogenic chemoreflex. Circulation 52:179-192, 1975 (reference 13)]

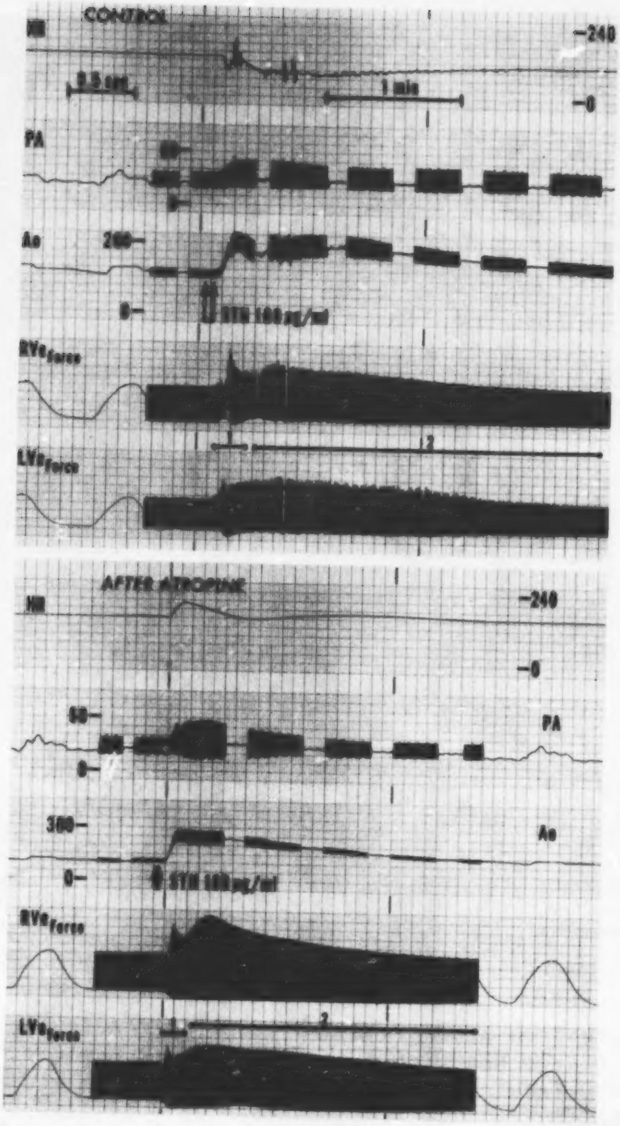

FIGURE 2. These two polygraphs illustrate typical responses to serotonin (STN) injected into the left atrium of an anesthetized dog. Intravenous administration of atropine 1 mg/kg eliminated the reflex bradycardia but not the marked hypertensive response. Positive inotropic responses occur in both ventricles. There is little change in pulmonary artery pressure. The numbered horizontal bars indicate two phases of the response: #1 is completely reflex, while the later and longer #2 represents some contribution from the release adrenal catecholamine stores (16). HR = Heart rate (beats/min). PA = Pulmonary artery pressure (mm Hg). Ao = Aortic pressure (mm Hg). RV and LV = Strain gauge records measuring contractile performance of the right and left ventricles, respectively. The paper speed for both records is indicated with a reference bar above. [Adapted by permission of the American Heart Association, Inc., from Urthaler F, Hageman GR, James TN: Hemodynamic components of a cardiogenic hypertensive chemoreflex in dogs. Circ Res 42:135-142, 1978 (reference 16)]

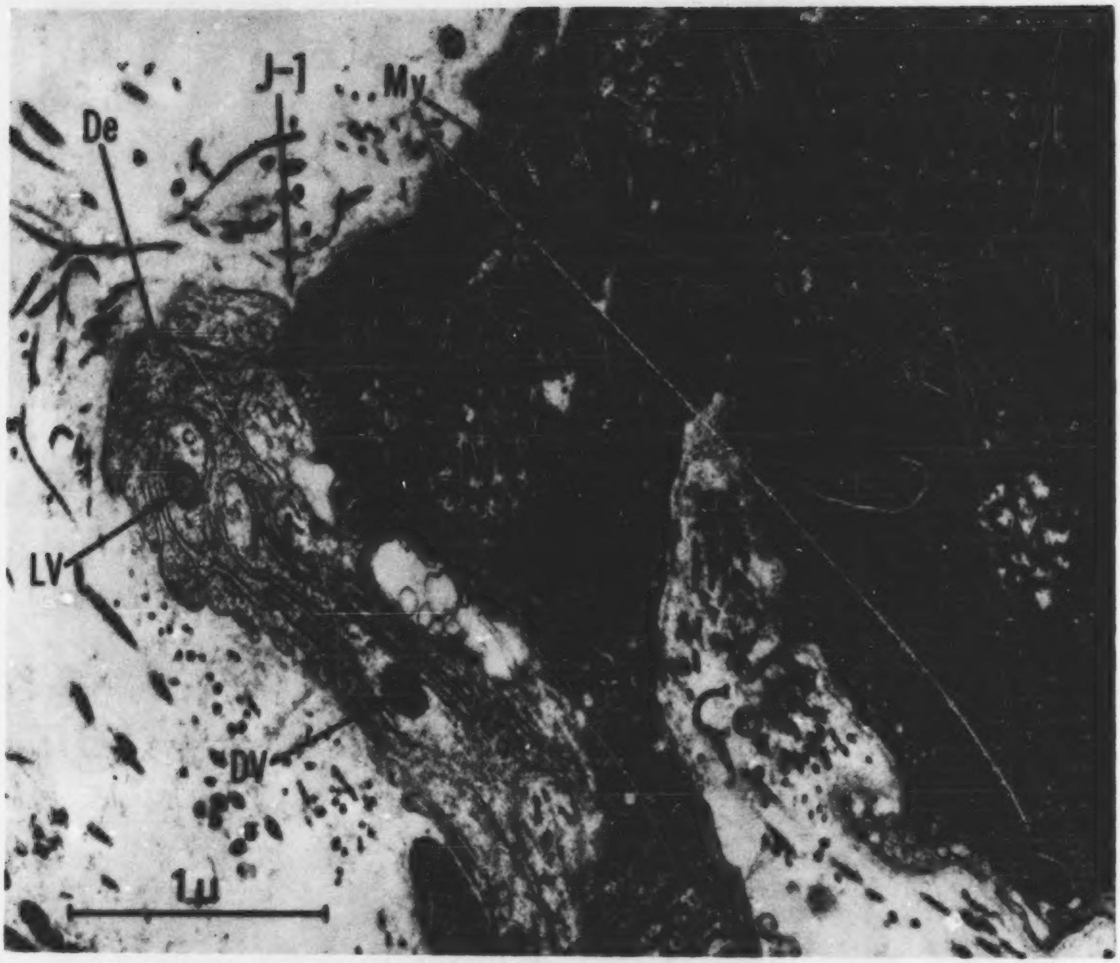

FIGURE 3. This electron micrograph depicts a neuromuscular junction in the eustachian ridge of the heart of a dog. DV = Dark vesicle. LV = Light vesicle. N = Nerve. Te = Tentacular extension of a myocyte. M = Mitochondria of the myocyte. My = Myofibrils. De = Desmosome. Co = Collagen. J-1 indicates one of two direct neuromuscular junctions found in this specimen; the other is not in this picture. [Reprinted with permission of the Yorke Publishing Company from Am J Cardiol 44:345-369, 1979 (reference 23)]

this knowledge, similar structures could be seen in the vicinity of other myocytes, although we were unable in that study to demonstrate another such clear example of direct junction. We suspect that much of the difficulty in making such anatomical demonstrations may be attributable to this perpendicular relationship between the nerve and muscle cells, thus making the fortuitous cut required to identify the junction a low order of probability. As a corollary, direct neuromuscular junctions may be more numerous within the heart than the present difficulty in demonstrating them would suggest.

Proceeding now from normal anatomy to structural abnormalities of the nerves and ganglia within the heart, these may be jointly considered as representing cardioneuropathy. Anatomically, this term embraces a very wide variety of histological changes, including noninflammatory degeneration, neuritis or ganglionitis, infiltrative disorders affecting nerves and ganglia (e.g., amyloidosis or leukemia), and hemorrhagic necrosis (7). For clearer discussion, the cardioneuropathies may be divided into those due to some known cause and part of a generalized or systemic disorder, and those in which the cardioneuropathy is primarily or exclusively an intracardiac disorder. This distinction between primary and secondary cardioneuropathy is thus according to whether the cause is exclusively or predominantly an intracardiac process or part of a more widespread disorder. Secondary cardioneuropathy can be part of some heritable neurodegenerative disease such as Friedreich's ataxia (24), or due to some generalized infection such as diphtheria (25) or a systemic illness such as polyarteritis nodosa (26).

Primary cardioneuropathy is a different problem, diagnosed principally by the absence of any clinical or postmortem evidence of extracardiac neural disease or any systemic illness or infection. This sort of exclusion is flawed by the realization that neural abnormalities in some other organs of the body may escape either premortem or postmortem detection unless especially sought. However, from what is known about a large number of cases of sudden and unexpected death (7-10), there is no question that nerves and ganglia within the heart were distinctly abnormal, and that no extracardiac disease was recognized.

Two forms of sudden death serve to illustrate primary cardioneuropathy. One group consists of patients who had in common a prolonged QT interval in their electrocardiograms (8). This group includes some patients who were born deaf as well as others who heard normally. The second group includes previously healthy subjects who either had some syncopal attacks prior to death or who died suddenly without any such preceding event (7,9,10). A few of these victims were known to have paroxysmal ventricular fibrillation, as were several of the patients with a long QT interval. In both groups there was focal degeneration of nerves within the ventricular myocardium, and even more prominently within the interatrial septum and in the vicinity of the sinus node, where ganglionitis was present in some hearts. However, there was a difference in the nature of the cardioneuropathy between the two groups: Patients with long QT intervals had more prominent ventricular neural lesions, while the hearts from sudden death victims in the second group had more distinctive ganglionitis near the sinus node. Given the difficulty in finding and identifying the neural lesions, it is almost impossible with present methods to quantify their exact prevalence and extent. Thus, the difference between prevalence of ventricular neuritic lesions and ganglionitis compared among these two groups is largely an interpretation by impression, based on reasonably careful assessment of hundreds of microscopic slides in each heart studied.

When the hearts of eight victims of sudden death who had been known to have long QT intervals were examined with a special search for neural lesions (8), these lesions were found to be abundant (figures 4-7). It seems likely that the ventricular neuropathy must contribute to disorganization of adrenergic neural influence on repolarization, since sympathetic nerves are by far the more numerous in the ventricles (4); however, no special effort was made to determine

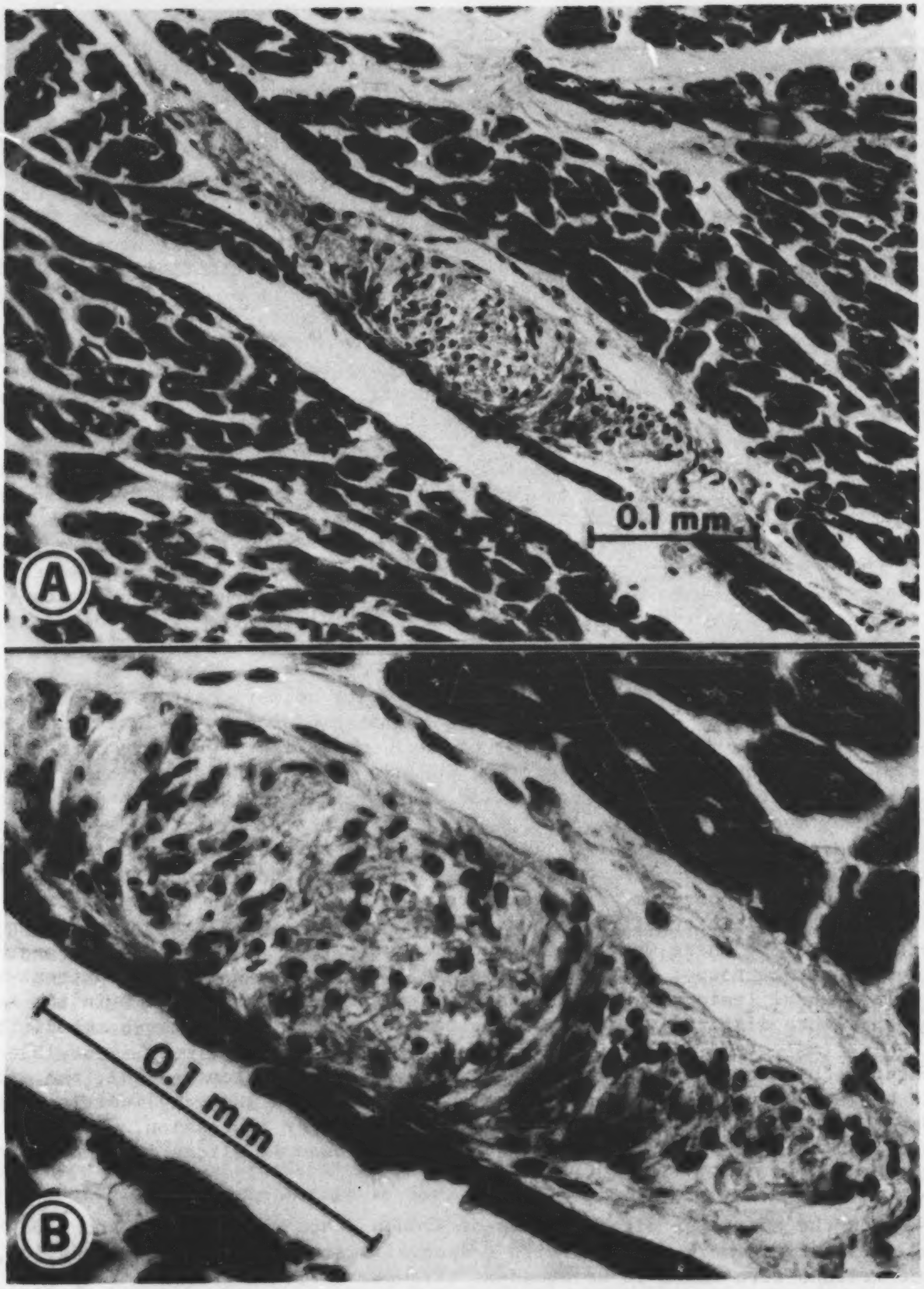

FIGURE 4. Inflammatory degeneration of a nerve within the ventricular myocardium of a patient dying with the long QT syndrome is shown here at two magnifications. [Reprinted by permission of the American Heart Association, Inc., from James TN, Froggatt P, Atkinson WJ, Lurie PR, McNamara DG, Miller WW, Schloss GT, Carroll JF, North RL: De Subitaneis Mortibus. XXX. Observations on the pathophysiology of the long QT syndromes with special reference to the neuropathology of the heart. Circulation 57:1221-1231, 1978 (reference 8)]

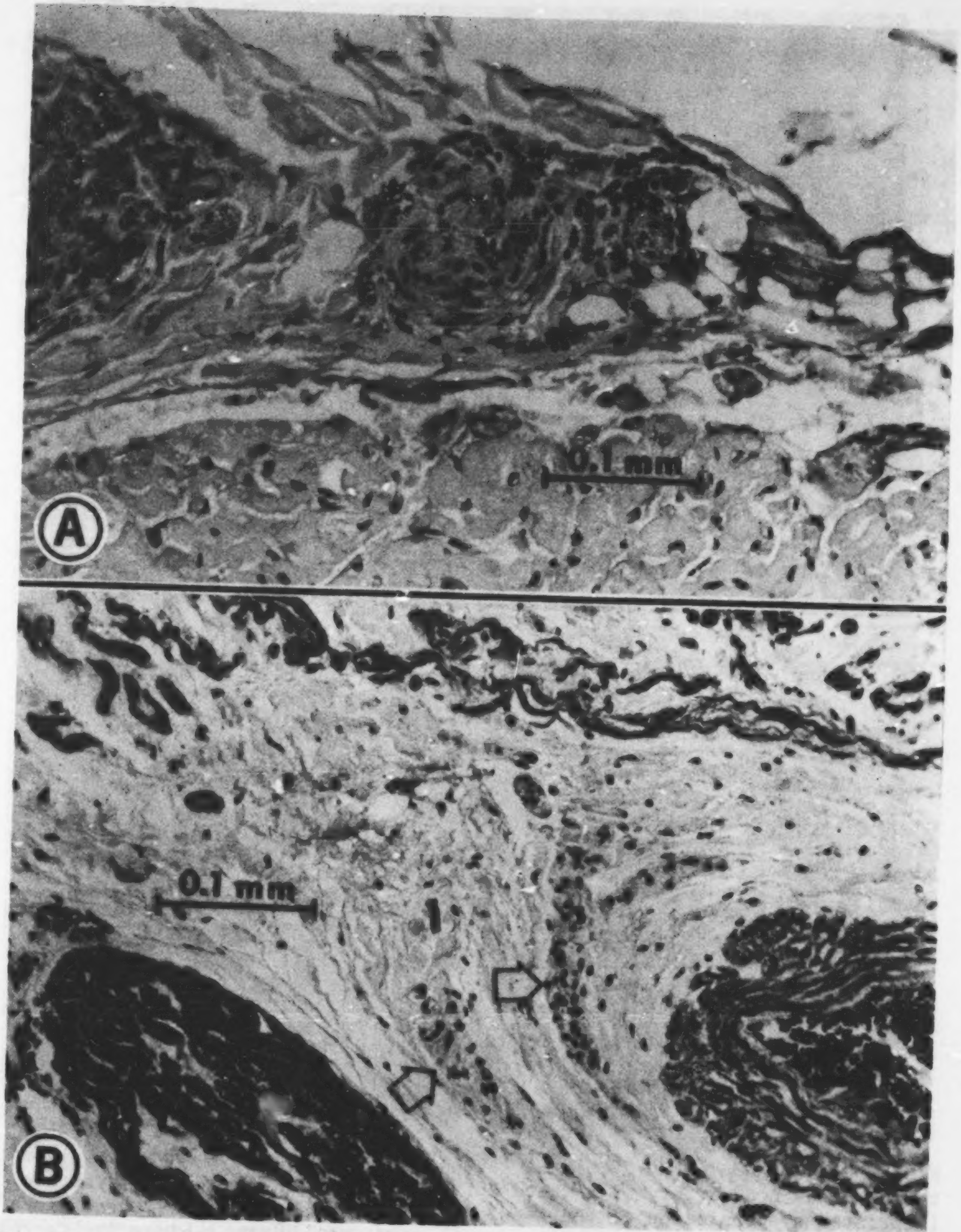

FIGURE 5. Two other examples of primary cardioneuropathy are shown here from a second patient dying with the long QT syndrome. In A (periodic acid Schiff stain) a ganglion near the sinus node contains leucocytic infiltration. In B two small nerves (arrows) are shown within scar tissue adjacent to the sinus node artery. [Reprinted by permission of the American Heart Association, Inc., from James TN, Froggatt P, Atkinson WJ, Lurie PR, McNamara DG, Miller WW, Schloss GT, Carroll JF, North RL: De Subitaneis Mortibus. XXX. Observations on the pathophysiology of the long QT syndromes with special reference to the neuropathology of the heart. Circulation 57:1221-1231, 1978 (reference 8)]

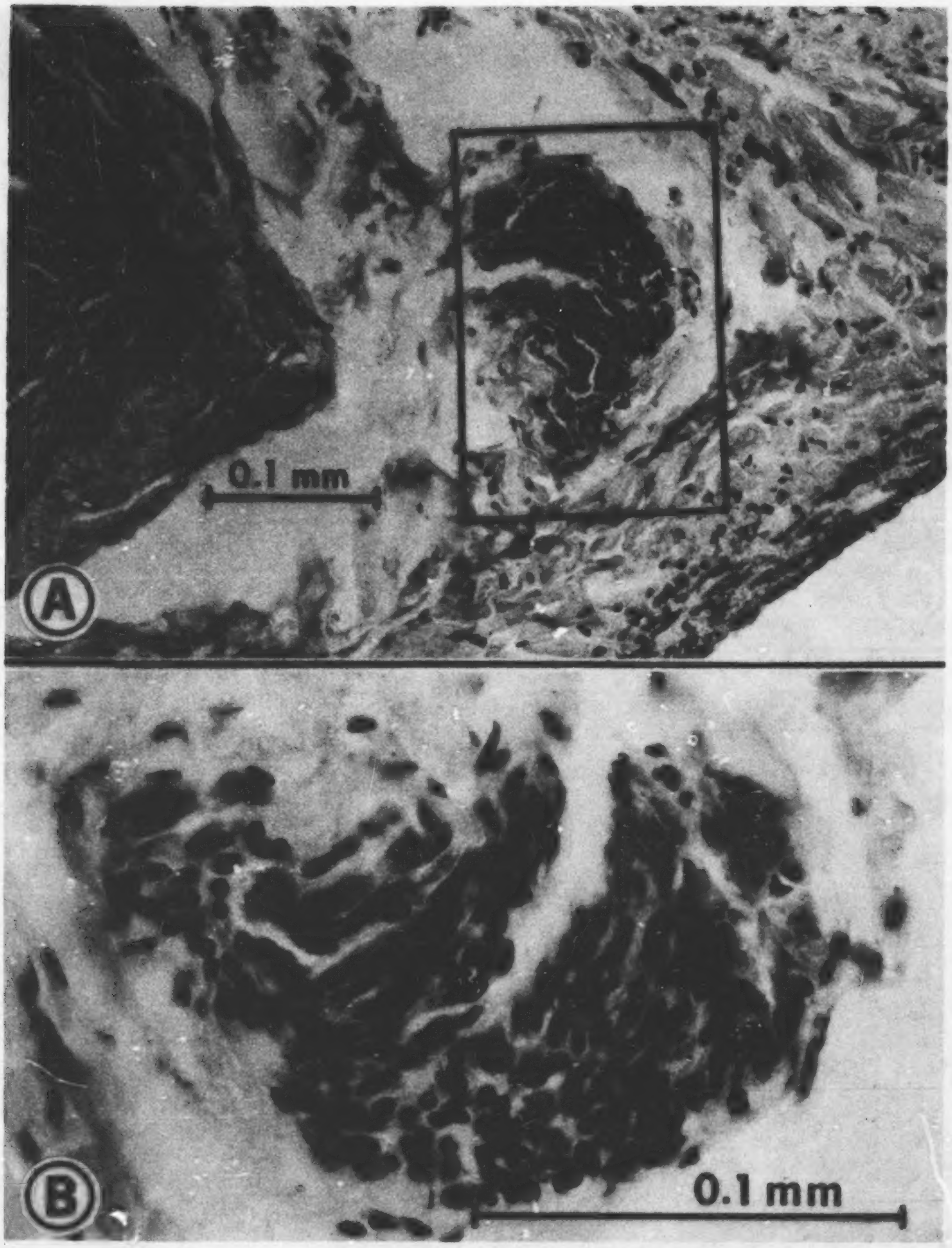

FIGURE 6. Marked leucocytic infiltration virtually destroys a nerve adjacent to the sinus node artery of a third patient dying with the long QT syndrome. [Reprinted by permission of the American Heart Association, Inc., from James TN Froggatt P, Atkinson WJ, Lurie PR, McNamara DG, Miller WW, Schloss GT, Carroll JF, North RL: De Subitaneis Mortibus. XXX. Observations on the pathophysiology of the long QT syndromes with special reference to the neuropathology of the heart. Circulation 57:1221-1231, 1978 (reference 8)]

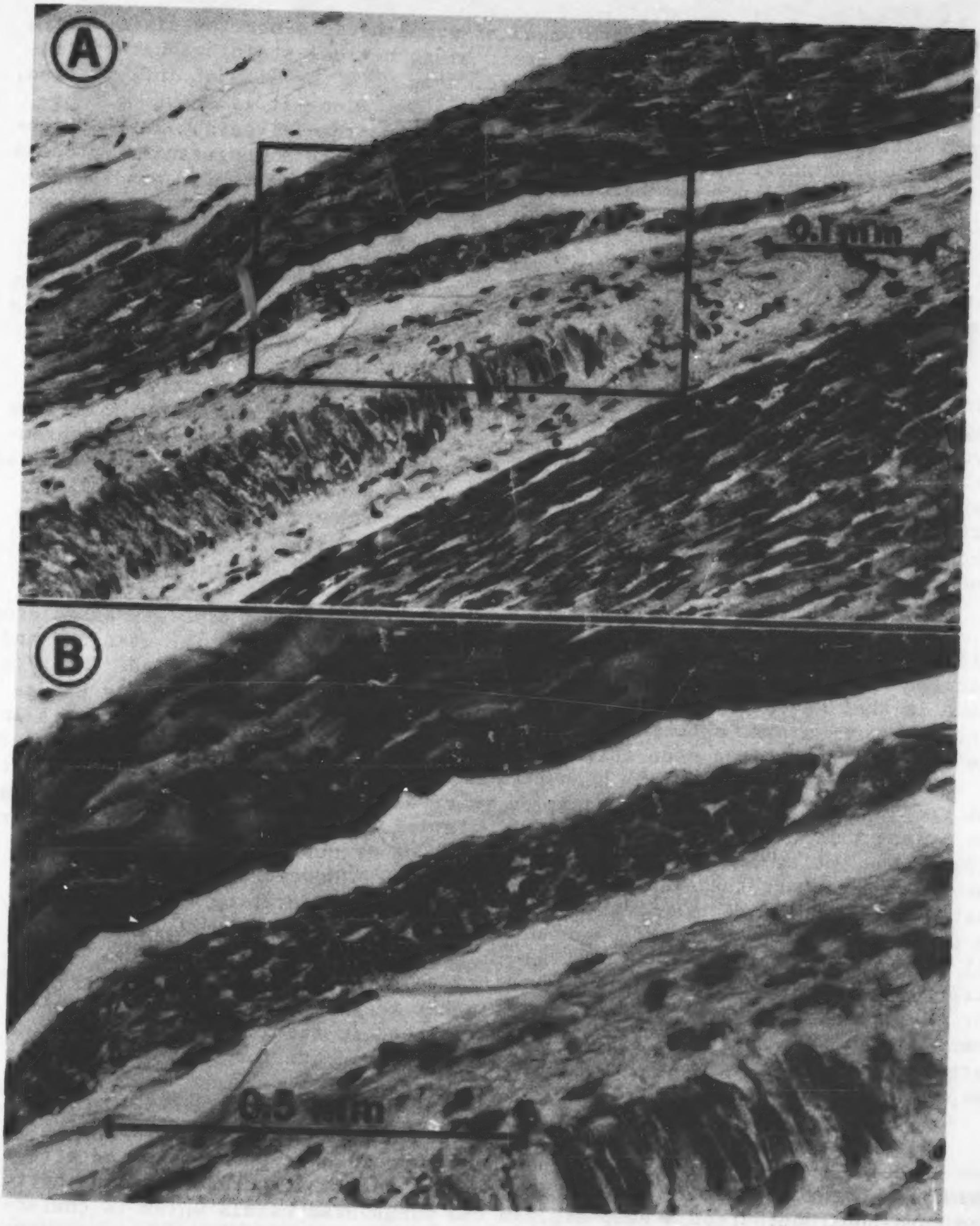

FIGURE 7. Another nerve filled with leucocytes is seen at two magnifications adjacent to a tangentially cut artery within the left ventricle of a fourth patient dying with the long QT syndrome. [Reprinted by permission of the American Heart Association, Inc., from James TN, Froggatt P, Atkinson WJ, Lurie PR, McNamara DG, Miller WW, Schloss GT, Carroll JF, North RL: De Subitaneis Mortibus. XXX. Observations on the pathophysiology of the long QT syndromes with special reference to the neuropathology of the heart. Circulation 57:1221-1231, 1978 (reference 8)]

whether these abnormal nerves were vagal or sympathetic since the disease seen in them could be anticipated to make their histochemical staining characteristics unreliable. Some nerves were scarred, some were the site of inflammation, and others had intermediate evidence of activity. Since it is known that patients with long QT intervals typically live for months or years with the problem, any neural contribution to its pathogenesis must be a longstanding process. Because of some evidence for heritable transmission of the long QT syndromes, it is possible that the cardioneuropathy is some heritable degenerative abnormality. However, the heritable hypothesis was originally based on a study of the associated deafness (27,28), and a different form of inheritance has been postulated for long QT syndromes seen in normally hearing subjects (28-31). It may now be appropriate to question (8) whether the long QT problem is heritable at all, or whether we have been misled by the frequently associated deafness. It is possible, for example, that the deafness and long QT interval have no etiological relationship, but that a chronic infection independently damages nerves within the heart. It is well known that certain communicable infections have an affinity for nerves or ganglia and can be very long lasting. For example, herpes simplex or herpes varicella-zoster both are known to follow familial distribution and can lie dormant years or decades before becoming clinically active again. This entire subject seems ripe for renewed inquiry to determine whether the cardioneuropathy is due to some infection, and if so, what can be done to treat or prevent it.

Ganglionitis of the heart as a single or predominant abnormality associated with sudden unexpected death is a recent discovery (9,10), and the etiology and specific functional significance of ganglionitis need to be clarified. What is known is that several young subjects who died suddenly and unexpectedly had as a sole or predominant abnormality the presence of a distinctive ganglionitis in the region of the sinus node (figures 8-10). Familial occurrence of sudden deat or ventricular arrhythmias has been present in some of these cases, but not (or not recognized) in others. In addition to viral infections which may lie dorman for a long time but eventually cause devastating destruction of one or more ganglia in the heart, one must also include some other chronic infections such as toxoplasmosis among the etiological possibilities. However, despite careful search for toxoplasma organisms, none have yet been identified in these hearts. On the other hand, viral particles in the vicinity of the ganglionitis (figure 11) have been found when carefully sought. As with most such anatomic demonstrations of viruses, there is no certainty that these were in fact responsible for the ganglionitis or, for that matter, that they caused any disease at all. However, the fact that they were sought and found in the location where they were suspected must be considered as supporting evidence for their being the responsible etiological agent.

Neural disease in the vicinity of the sinus node can be either vagal or sympathetic, and destruction of either nerves would have an anticipated effect opposite to the other. If some or most of the nerves affected are sympathetic, the cardioneuropathy could help explain the sinus bradycardia which is characteristic of most patients with the long QT syndromes (8,28,29). These patients also have focal thickening of their sinus node artery (28,29), which may further distort sinus pacemaking, and one must consider whether the focal fibromuscular dysplasia was secondary to longstanding local neuropathy. In view of the extent and widespread distribution of the neural lesions in the heart and the paucity

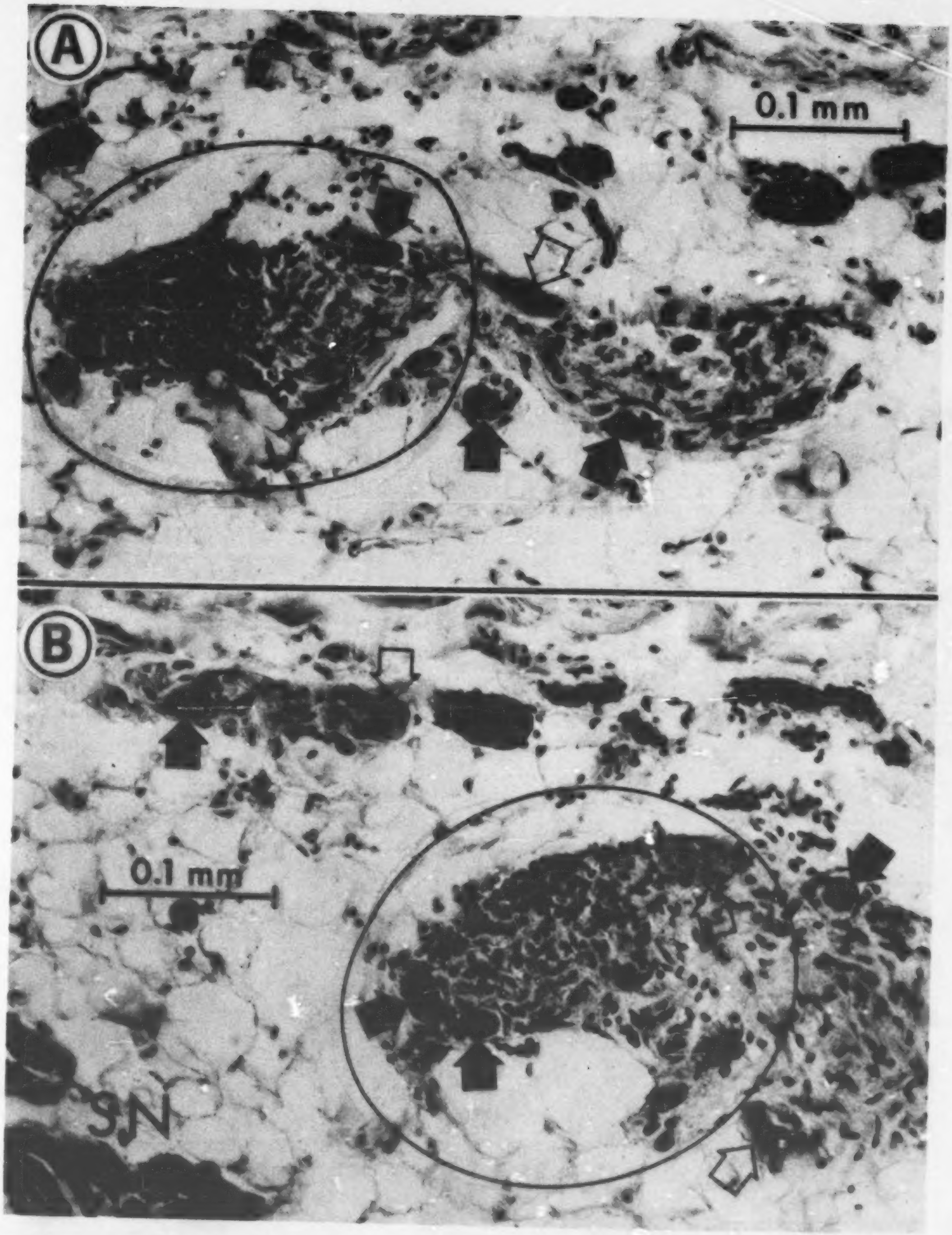

FIGURE 8. Another form of primary cardioneuropathy is illustrated with ganglionitis depicted here from the heart of a young woman dying suddenly and unexpectedly. Inflammation is encircled in these photomicrographs from adjacent sections. The black arrows mark shrunken or pyknotic neurons, while the white arrows mark discolored and swollen or enlarged neurons. These sections are from the margin of sinus node. [Reprinted with permission of the American College of Physicians from Ann Intern Med 91:727-730, 1979 (reference 9)]

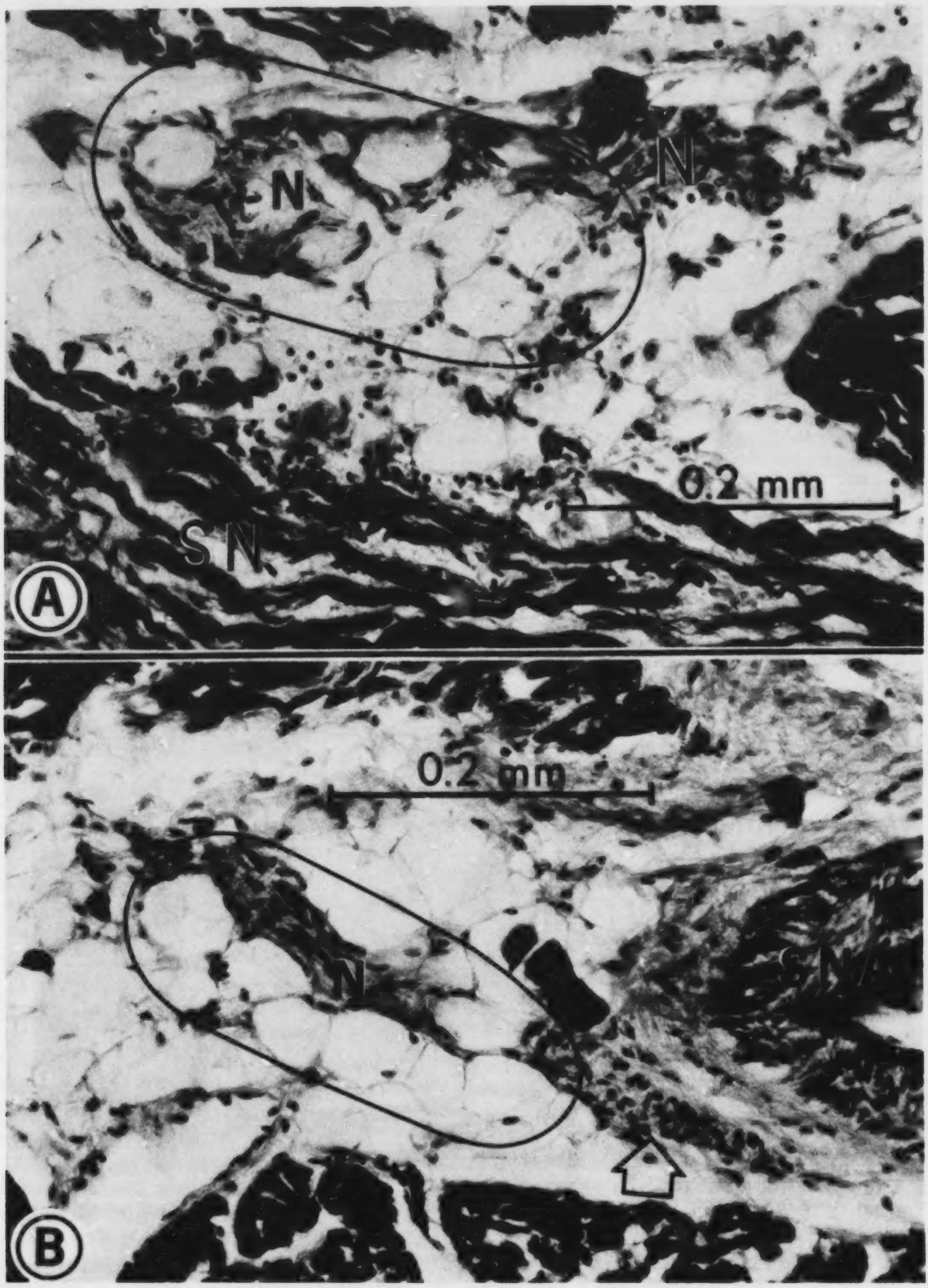

FIGURE 9. Vesicular degeneration of two nerves (encircled) near the sinus node (SNA) are shown from the heart of the same victim of sudden unexpected death shown in figure 8. N marks the nerves and SN a portion of sinus node. The white arrow indicates cicatricial damage to one portion of the nerve. [Reprinted with permission of the American College of Physicians from Ann Intern Med 91:727-730, 1979 (reference 9)]

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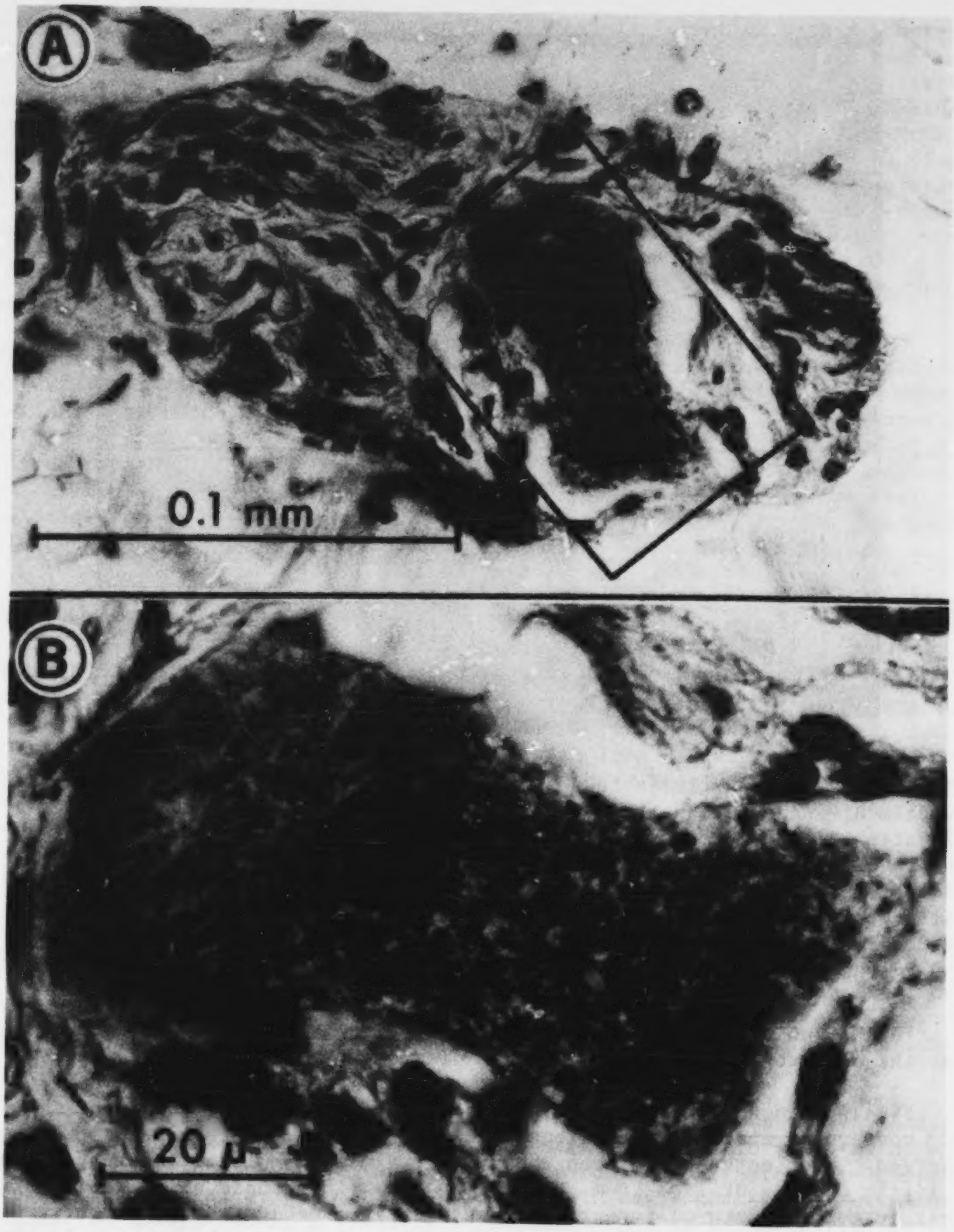

FIGURE 10. Nissl degranulation of one neuron is illustrated here at two magnifications. White arrow marks a possible inclusion body. This ganglion is adjacent to the sinus node of the victim of sudden death identified in figures 8 and 9. [Reprinted with permission of the American College of Physicians from Ann Intern Med 91:727-730, 1979 (reference 9)]

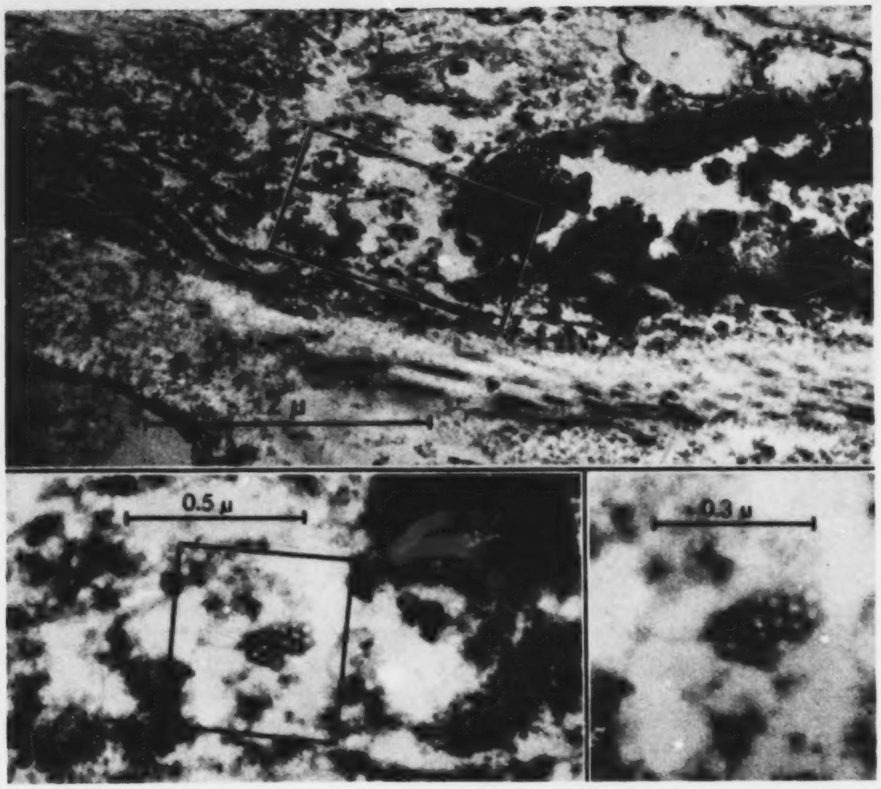

FIGURE 11. Viral particles found at the margin of sinus node from the heart of a victim of sudden unexpected death are illustrated here at three different magnifications. The specimen for this electron microscopic study by Dr. Kikuko Imamura was excised from the sinus node identified within a paraffin block from which previous histological sections had permitted examination of the ganglionitis near the sinus node. (Reprinted with permission of the American Clinical and Climatological Association from reference 10)

of arterial lesions, it seems improbable that it was the other way around, i.e., that the arteriopathy caused the neural disease.

Finally, in this description of anatomical findings, some comment should be made concerning the interpretation and method of study of the specific changes observed. When nerves have been entirely or almost completely destroyed, there is little one can utilize from examination of a microscopic slide to identify their former presence or integrity. Fortunately, the length of most nerves lends itself to sequential study in serial sections, and this method is invaluable in assessing whether a given degenerative or inflammatory focus in the heart was actually within a nerve or not. Conversely, examination of normal nerve in one section can be coupled with scrutiny of that same region in multiple adjacent sections, where surprising focal disease may be uncovered. In addition to leucocytic infiltration and neurofibrillar degeneration for identification of neural disease, a third abnormality to be sought is vesicle formation within a nerve (figure 9). Ganglia can be recognized as abnormal by certain changes such as infiltration with leucocytes (figures 5A and 8). More subtle but still distinctive changes within ganglia include Nissl degranulation, heterochromic staining properties of multiple neurons within a ganglion, and varied size of neurons, ranging from enlargement (swelling) to shrinkage (pyknotic degeneration) (figures 8 and 10). With the Goldner trichrome stain the polychromasia of degenerating neurons of individual ganglia is sometimes a striking finding, most often combined with Nissl degranulation or anisocytosis of the neurons.

FUNCTIONAL SIGNIFICANCE OF CARDIONEUROPATHY

Neural lesions within the heart will predictably influence its inotropic responses or perhaps change the pattern of coronary flow, since both of these functions respond to neural stimuli. However, this discussion concentrates on electrophysiological responses which may be anticipated in the presence of cardioneuropathy. These include the distortion of myocardial repolarization, cardiac impulse formation (normal and ectopic), and AV conduction. Atrial repolarization is particularly influenced by the vagus nerve (32), while adrenergic innervation has a more important effect on ventricular repolarization (33).

Paramount among clinical examples of significant distortion of ventricular repolarization as a major problem are the patients with so-called long QT syndrome. Although first recognized among children born deaf (27) and later among ones with normal hearing as well (30,31), it was some years before evidence was presented to indicate that sudden deaths observed among such patients were in fact cardiac events (28,29). It is now generally believed that both the fainting spells and sudden deaths among patients with long QT intervals are due to ventricular arrhythmias, and many such examples have been documented. Based on experimental evidence initially obtained in dogs (34), the distorted pattern of ventricular repolarization in patients with long QT intervals is probably due to asymmetrical adrenergic neural influence on the ventricular myocardium. This interpretation is supported by the observation that anesthetic blockade of the stellate ganglion (usually the left) can normalize ventricular repolarization, and unilateral ganglionectomy can abolish the syncopal attacks and ventricular arrhythmia (35). However, the cause of adrenergic neural asymmetry in these patients was not determined. Furthermore, some patients fail to benefit from anesthetic blockade of the stellate ganglion so that the success rate for such blockade may be less than presently thought, knowing that physicians are less apt to report or publish their failures.

Slow heart rates are known to predispose to escape beats and arrhythmias; therefore, the sinus bradycardia of patients with long QT intervals may be an important factor in the pathogenesis of their paroxysmal arrhythmias. However, there is experimental evidence that AV junctional escape rhythms are even more dependent on normal adrenergic neural innervation than is sinus rhythm (22). Thus, if faulty adrenergic innervation is held responsible for the abnormal ventricular repolarization and, at least in part, for the sinus bradycardia, it may be equally dangerous in causing the most effective alternative pacemaker in the heart to be halting or possibly to fail. In other words, many of the electrophysiologic unstabilizing processes seen in patients with long QT syndromes may have their origin in neural disease of the type now demonstrated to be present (8).

Along with these more or less direct results of faulty innervation of the heart caused by any cardioneuropathy, we must add consideration of how such disease may alter the anticipated effects of cardioactive drugs or other circulating substances. For example, quinidine has a powerful anticholinergic influence when examined by selective perfusion of the canine sinus node through its nutrient artery, and this effect is manifest in concentrations comparable to ones achieved in clinical therapy (36). Digitalis not only has a significant antiadrenergic effect (37), but the "ventricular" arrhythmia produced with toxic concentrations of digitalis in the dog can be abolished by selective administration of either acetylcholine or physostigmine into the AV node artery (38). There are still other widely used medications which have varying relationships to activity of the vagus and sympathetic nerves, but many victims of sudden unexpected death are not taking such medications. Some of them drink ethanol, however, and how this could alter neural responsiveness even in the normal heart but more so if cardioneuropathy were present is considered below.

Ethanol itself has surprisingly little direct effect on either the sinus node or the AV junction (node and His bundle) of the dog when administered selectively into these regions via local nutrient arteries (39). This absence of effect persists even with enormous concentrations much beyond those to be anticipated in man. On the other hand, in every person who drinks ethanol the first breakdown product is acetaldehyde, which is the normal result of action by alcohol dehydrogenase in the liver. The production of acetaldehyde from ethanol is a biochemical transformation which continues to function even very late in the presence of cirrhosis or other liver disease. Thus, the chronic alcoholic patient continues to transform ethanol to acetaldehyde almost as long as the liver functions at all. Acetaldehyde is normally degraded to carbon dioxide and water by aldehyde mutase. It is the pharmacological blockade of this transformation by disulfiram which is responsible for its successful use in the treatment of chronic alcoholism. When disulfiram (Antabuse®) blocks the action of aldehyde mutase, acetaldehyde steadily accumulates in the body and causes many disagreeable symptoms. These include vasomotor flushes, tachycardia and hypertension, all mediated at least in part by acetaldehyde (40).

In contrast to the absence of significant direct effect of ethanol on the sinus node or AV junction, acetaldehyde has a profound influence (39,41) due almost entirely to the brisk release of local norepinephrine stores (figure 12). This property is shared by some other short-chain aliphatic aldehydes, such as butyraldehyde and propionaldehyde. On the other hand, different aliphatic aldehydes such as formaldehyde and glutaraldehyde caused marked prolongation of the QT interval rather than tachycardia and, eventually, the spontaneous appearance of ventricular arrhythmias (41). Either of these latter substances may be considered as an abnormally toxic agent, but it should be kept in mind that formaldehyde is one step in a number of "physiological" biochemical processes in vivo, just as acetaldehyde accumulates rapidly and progressively in any one who drinks ethanol. A particularly intriguing consideration concerns the potential interplay of any of the aldehydes with adrenergic neural events, with acetaldehyde releasing norepinephrine while formaldehyde acts on ventricular repolarization

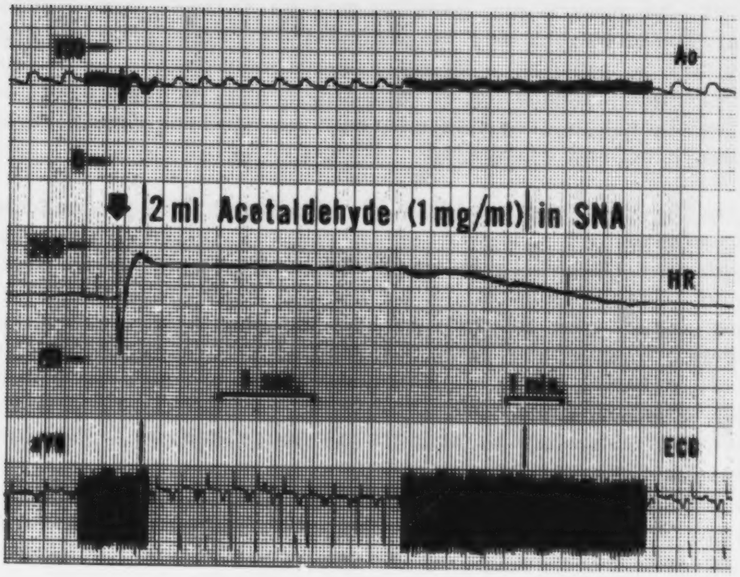

FIGURE 12. This polygraph illustrates the marked positive chronotropic action of acetaldehyde selectively perfused into the sinus node artery (SNA) of an anesthetized dog. Transient bradycardia during any injection directly into the sinus node artery is a characteristic response with this method. Ao = Aortic pressure. HR = Heart rate. ECG = Electrocardiogram. [Adapted with permission of the C. V. Mosby Company from James TN, Bear ES: Effects of ethanol and acetaldehyde on the heart. Am Heart J 74:243-255, 1967 (reference 39)]

in a fashion very similar to experimentally produced adrenergic neural asymmetry (34) by prolonging the QT interval. When chronic alcoholism reaches the stage of causing important malnutrition, then thiamine deficiency and beri-beri add still another dimension to possible cardioneuropathy. In view of the fact that drinking ethanol is such a longstanding and widespread practice over most of the world, the possible role which acetaldehyde may play in adding to the electrical instability of the heart, particularly if cardioneuropathy is present, deserves further investigation.

SUMMARY

Neural control of the heart furnishes one of the most important means of stabilizing its electrical activity. Diseases of the nerves and ganglia within the human heart can be the basis for serious distortions of impulse formation, conduction, and repolarization. Two special features of normal neuroanatomy of the heart described in this report include an intracardiac chemoreceptor which receives its blood supply from the proximal left coronary artery, and the electron microscopic appearance of a direct neuromuscular junction. A powerful cardiogenic hypertensive chemoreflex elicited by serotonin (a naturally occurring substance carried almost entirely by the platelets) is briefly described.

Diseases of the nerves and ganglia may be jointly considered as cardioneuropathies. Cardioneuropathy may be secondary to some generalized or systemic disease, or it may be primary. Primary cardioneuropathy is diagnosed when the only evidence of such disease is within the heart, whatever the etiology. Examples of human cardioneuropathy associated with various forms of sudden unexpected death are illustrated and discussed.

The concluding section considers the functional significance of cardioneuropathy based on experimental studies in the dog in which selective perfusion of the sinus node or the AV junction (node or His bundle) is performed to assess the influence of various substances of neural responsiveness of these particularly important structures in the heart. Because alcoholism is sometimes associated with sudden unexpected death, experiments illustrating the negligible direct effect of ethanol but the profound effect of certain aldehydes are described. Since ethanol is normally degraded to acetaldehyde within the human liver, the electrophysiological effects of acetaldehyde (which actively releases norepinephrine) receive special attention.

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PLASMA LIPIDS AND LIPOPROTEINS AND DEGREE OF ATHEROSCLEROSIS OF THE AORTA AND CORONARY ARTERIES IN CASES OF SUDDEN CARDIAC DEATH

S. G. Aptekar, A. M. Vikhert, E. E. Matova, and I. E. Galakhov

ABSTRACT. This study involved 114 men who died suddenly due to coronary heart disease (CHD). Hypercholesterolemia was found in 43 percent of the cases. A lowering of the high-density lipoprotein cholesterol content was observed in 75 percent of the victims. Thus, hypo-α-cholesterolemia was more frequently correlated with CHD than was hypercholesterolemia. Type IIa hyperlipoproteinemia was observed in the majority of persons who died suddenly; type IIb was observed in approximately one-third of the cases. The area of lipidosis in the aorta did not correlate with the plasma cholesterol level. The cholesterol content in the fatty streaks in the aorta was significantly higher in persons with a high plasma cholesterol level. The area of aortic raised lesions was significantly greater in persons with a high plasma cholesterol level than in persons with a low cholesterol level. The cholesterol content of the most severe lesions did not correlate with the plasma cholesterol level. In persons with high plasma cholesterol levels, isolated coronary artery stenoses were encountered less frequently, while multiple stenoses were encountered more frequently than in persons with low plasma cholesterol levels. In persons with high plasma cholesterol levels and coronary artery stenosis, the constriction of the vessel was always greater than 50 percent of its lumen, whereas in persons with low blood cholesterol levels stenosis did not exceed 50 percent of the vessel lumen in 10.4 percent of the observations.

INTRODUCTION

A number of long-term prospective studies have confirmed the independent role of hypercholesterolemia as a risk factor in coronary heart disease (1,2).

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Such indexes as hypertriglyceridemia (3) and a low level of high-density cholesterol lipoproteins or α-cholesterol in the blood (4,5) are also related to the frequency of CHD.

However, the status of lipid metabolism in victims of sudden death due to CHD has not been sufficiently studied. The relationship between plasma lipid levels and the degree of atherosclerosis in the aorta and coronary arteries in cases of sudden death has also never been studied. The results of such investigations may be significant for both evaluating the risk of sudden cardiac death and for determining the role of lipids in the pathogenesis of atherosclerosis in general and coronary atherosclerosis in particular.

MATERIALS AND METHODS

The present study covered 114 men who died suddenly of CHD. The cause of death was documented by detailed autopsies, as well as by macroscopic and microscopic studies of the heart and other internal organs. The age of the victims ranged from 24 to 65 years.

During autopsy, blood for testing was taken from the vena cava superior and collected in test tubes containing dry EDTA; it was then agitated carefully, and the plasma was separated by centrifugation. Plasma taken no more than 3 hours after death was used for the lipoprotein studies. The content of plasma cholesterol was determined by the method of Abell et al. (6), and triglycerides were determined by the method of Carlson (7). Very low-density lipoproteins (VLDL) were separated by applying a layer of 0.15 M NaCl onto the blood plasma with centrifugation for 18 hours at 105,000 g at 10° C in a 40.3 rotor in a type L-5-65 (Beckman) ultracentrifuge. The top fraction, containing the VLDL, was discarded. The concentration of cholesterol in high-density lipoproteins (HDL) was determined in the infranatant left after removal of the VLDL and after precipitation of low-density lipoproteins (LDL) with heparin in the presence of manganese ions. The content of LDL cholesterol was calculated on the basis of the difference in its content in the summary fraction of LDL and HDL and the content of cholesterol in the HDL.

In order to phenotype the hyperlipoproteinemias (HLP), the plasma lipoproteins were separated into individual fractions by the disk electrophoresis method in polyacrylamide gel. The fractions produced after separation were measured quantitatively on a QM-3 densitometer manufactured by Opton (Federal Republic of Germany).

The control was blood plasma obtained from male primary donors 20-39 years of age; analogous indexes of lipid metabolism were determined by the same methods as were used on the blood from the sudden death victims. For the 40- to 59-year-old age group, the control data used were those published previously by Suchkova et al. (8) from a study of men living in one neighborhood in Moscow.

We determined the cholesterol content in the aorta in 53 cases of sudden death. The macroscopically unaltered sections of the intima, fatty streaks, and fibrous plaque were studied separately. Sections of the aorta were ground and then extracted by the method of Folch et al. (9). In the lipid extract produced, the specific color reaction with Liberman-Burchard reagent was used to determine the concentration of cholesterol, expressed in mg cholesterol per 100 mg of dry, degreased aortic tissue.

The degree of atherosclerosis of the thoracic and abdominal aorta was studied by visual-planimetric evaluation of atherosclerotic changes based on the ratio of areas of damaged intima to the total area of the vessel. This method was recommended by a group of World Health Organization (WHO) pathologists for epidemiology study of atherosclerosis. The area was occupied by fatty streaks and elevated atherosclerotic changes (in the total area of the fibrous plaque complicated and calcified).

The degree of coronary artery atherosclerosis was estimated from the presence of stenosis in the lumen. Stenosis of less than 50 percent of the lumen (subgroup I) and more than 50 percent of the lumen (subgroup II) were distinguished.

RESULTS

Plasma Lipids and Lipoproteins in Sudden Cardiac Death Victims

The distribution by age of persons who died suddenly of CHD is shown in table 1. As the table shows, the largest group was the 40- to 49-year-old age group, followed closely by the 50- to 65-year-old age group, and more distantly by the 30- to 39-year-old age group. The smallest group was the 20- to 29-year-old age group. These figures are close to those found elsewhere in the literature.

The distribution of persons by age and plasma cholesterol level is shown in table 2. The level of plasma cholesterol in sudden death victims varies from the normal and ranges from low to high. We therefore divided all victims into two subgroups based on their plasma cholesterol level: those with plasma

TABLE 1. Percent Distribution of Sudden Death Victims According to Age

Age (years)	No. of victims	% of total victims
20 - 29	3	3.5
30 - 39	14	12.3
40 - 49	49	43.3
50 - 65	47	41.2
	114	100.3

TABLE 2. Plasma Lipids and Lipoproteins in Sudden Death Victims (M ± m)

Group	No.	Cholesterol (mg%) TC	α-C	β-C	α-C, % of TC	$\frac{\alpha\text{-C}}{\beta\text{-C}} \times 10$	Triglycerides (mg%)	Type of hyperlipoproteinemia (% of number of cases in group) Normal	IIa	IIb	IV
1. 20-29 years old											
Plasma C less than 250 mg%	4	150	50.8	105	34	4.8	63	100	-	-	-
2. 30-49 years old											
a) Plasma C less than 250 mg%	37	202 ± 4.8	35.7 ± 3.5	134 ± 7.5	17.7	2.7	164 ± 23	72	-	-	28
b) Plasma C more than 250 mg%	26	315 ± 17	36 ± 4.2	192 ± 13	11.4	1.9	176 ± 22	-	69	31	-
3. 50-65 years old											
a) Plasma C less than 250 mg%	24	202 ± 9	33 ± 3.7	120 ± 14	16.3	2.8	110 ± 9.6	94.7	-	-	5.3
b) Plasma C more than 250 mg%	23	318 ± 13	33.4 ± 5.9	187 ± 10	10.5	1.8	139 ± 17	-	63.6	36.4	-
4. Persons with myocardial infarction											
a) Plasma C less than 250 mg%	6	187	44.3	118.8	23.7	3.7	145.3	83.3	-	16.7	-
b) Plasma C more than 250 mg%	5	301	20.8	204	6.9	1.0	196	-	40	40	20

$p < 0.001$ in comparison of cholesterol levels for groups 2, 3, and 4 with groups of healthy persons of the same ages.
C = Cholesterol. TC = Total cholesterol. α-C = Cholesterol in high-density lipoproteins. β-C = Cholesterol in low-density lipoproteins.

cholesterol less than 250 mg%, and those with more than 250 mg%. The figure 250 mg% was taken as the mean high-normal limit of plasma cholesterol content, which, according to the literature, is characteristic for persons over 30 years of age. This quantity is close to that which we found in Moscow residents of a corresponding age. Due to the low number of victims in the 30- to 39-year-old age group, we decided for analysis purposes to combine this group with the 40- to 49-year-old age group.

Of the total number of persons (114) who died suddenly from CHD, autopsy revealed myocardial infarction in 11. We analyzed this group both as a part of the corresponding age groups, and as an independent group. The results of this analysis are also shown in table 2.

In developing phenotypes for hyperlipoproteinemia, we accepted 250 mg% as the high-normal limit for cholesterol, and 140 mg% for triglycerides. In type IIa HLP, the plasma cholesterol level exceeded 250 mg%, and the content of triglycerides was normal. Electrophoresis showed a sharp beta band, with the pre-beta fraction diffuse or absent.

Type IIb HLP was manifested by a plasma cholesterol level over 250 mg%, and triglycerides were over 140 mg%. Electrophoresis showed a clear pre-beta fraction band.

Type IV HLP was characterized by a normal or slightly elevated plasma cholesterol level, with a high level of triglycerides (over 140 mg%). Electrophoresis showed a clear separate pre-beta lipoprotein band. Diagnosis of types I and V was based on the presence of chylomicrons when the blood plasma was allowed to stand. We did not diagnose type III HLP, which can be found only by performing paper electrophoresis ("wide beta").

As can be seen from table 2, the total blood cholesterol content of persons in both age groups (30-49 and 50-65 years) was similar and almost identical; however, the percentage of persons with a high level of blood plasma cholesterol was greater in the older age group. In the 30- to 49-year-old age group, hypercholesterolemia was found in 41 percent of the sudden death victims, while it was found in 51 percent of the 50- to 65-year-old age group. Attention is also drawn to the lower content of triglycerides in the blood plasma of persons in the older age group. In accordance with this, type IV HLP is almost not seen in this age group. The percentage of persons with HLP is similar in both age groups, and type IIa according to Fredrickson's classification is predominant. We should emphasize that HLP was not found in 44 percent of the sudden death victims. Thus, in spite of the fact that HLP was frequently seen among persons who died suddenly of coronary heart disease, it was certainly not an obligatory manifestation of this disease.

Attention is drawn to the data on cholesterol content in various classes of plasma lipoproteins. First, we note the low content of HDL cholesterol (α-cholesterol) in persons of both age groups, with the absolute value of α-cholesterol concentration similar in the same groups with high and low levels of total blood cholesterol. However, the ratio of this index to the LDL cholesterol content was less in the subgroups with a high total cholesterol content in the blood plasma. We should emphasize that the level of α-cholesterol was not decreased in all persons; in 25 percent of the cases this index was normal. However, for the groups as a whole, the level was always lower than in the control (see table 3).

In both subgroups with a high level of total blood plasma cholesterol, there was a significantly increased content in the cholesterol of atherogenic low-density lipoproteins. Also, the coefficient reflecting the ratio of HDL to LDL was lower in these subgroups. This ratio was also lower in the subgroups with a normal level of total blood plasma cholesterol, although to a lesser extent than in persons with hypercholesterolemia. This coefficient was always lower in sudden death victims than in the corresponding ages of the healthy population. The only exception was the very small group of young persons (20-29 years, four cases), in which both the total cholesterol content in the blood plasma and its concentration in the different classes of lipoproteins were normal.

Analysis of the data from studying the lipid content in the blood plasma of sudden death victims with myocardial infarction showed that: Of 11 cases, the blood plasma cholesterol level was normal in 6, and hypercholesterolemia was found in 5, or 45 percent, of the cases. The mean cholesterol level in the blood plasma with hypercholesterolemia was 301 mg%. Hypercholesterolemia was combined with hypertriglyceridemia in two cases (type IIb HLP), while in one case we assumed type IV HLP. The content of α-cholesterol was low and in only one case was this index normal. In addition to this, there was an increase in the content of LDL cholesterol.

Morphometry of Aortic and Coronary Artery Atherosclerosis and Biochemical Data in Sudden Cardiac Death Victims

Results of data analysis in the study on the degree of atherosclerosis in the thoracic and abdominal aorta are presented in table 4. This analysis

TABLE 3. Blood Plasma Lipids and Lipoproteins in Control, Essentially Healthy Men of Various Ages Living in Moscow (M ± m)

Age group	No.	Cholesterol (mg%)			α-C, % of TC	$\frac{\alpha\text{-C}}{\beta\text{-C}} \times 10$	Triglycerides (mg%)
		TC	α-C	β-C			
20-29 years	357	193 ± 2.6	52 ± 1.12	125 ± 1.90	27	4.2	101 ± 3.9
30-39 years	151	220 ± 4.8	53 ± 2.60	143 ± 3.80	24	3.7	105 ± 5.3
40-49 years*	323	216 ± 1.8	56 ± 1.00	140.9 ± 2.0	26	4.0	100

*Data of Suchkova et al. (8) obtained by examination of men living in one area of Moscow. See table 2 for designations of abbreviations.

TABLE 4. Area of Atherosclerotic Lesions of the Thoracic and Abdominal Aortas as Percent of the Intimal Surface

Age (years)	Group	Thoracic aorta		Abdominal aorta	
		Fatty streaks	Raised lesions	Fatty streaks	Raised lesions
30 - 49	I	8.9 ± 0.9	7.0 ± 1.5*	8.9 ± 0.8*	23.0 ± 1.4*
	II	9.0 ± 0.7	11.5 ± 1.7*	6.7 ± 0.8*	25.8 ± 1.7
	III	13.4 ± 0.4	3.5 ± 0.1	17.5 ± 0.4	9.2 ± 1.0
50 - 65	I	7.7 ± 0.8	15.8 ± 1.6**	4.3 ± 0.6	34.6 ± 1.5**
	II	7.9 ± 0.5	25.0 ± 1.4**	3.9 ± 0.7	45.8 ± 1.6**
	III	7.2 ± 0.5	14.9 ± 0.9	6.5 ± 0.4	30.6 ± 0.8

*$p < 0.05$ for comparison of groups I and III, as well as II and III.
**$p < 0.05$ for comparison of groups I and II, as well as II and III.

was performed on the same subgroups used in the analysis of blood plasma lipids. The first group included cases with a blood plasma cholesterol level of not over 250 mg%; the second group included cases with a level over 250 mg%; and the third group was a control, consisting of essentially healthy persons living in Moscow who died from trauma (10).

As can be seen in this table, the area of fatty streaks, both in the thoracic and abdominal aorta, fails to correlate with the level of blood plasma cholesterol. The area of fatty streaks in the aorta decreases with increasing age. In essentially healthy persons (group III), the fatty streaks in the aorta occupy a somewhat greater area than in sudden death victims, particularly in the 30- to 49-year-old age group.

The situation is quite different in the area of raised lesions. In the 30- to 49-year-old age group, we already see a tendency toward predominance of raised lesions in persons with a higher level of blood plasma cholesterol (group II). This tendency is seen particularly clearly in the 50- to 65-year-old age group, where the area of raised lesions in the thoracic and abdominal aorta in persons with high blood plasma cholesterol is significantly greater than in persons with a low blood cholesterol level. In essentially healthy persons, raised lesions in the aorta occupy a smaller area than in sudden cardiac death victims; these differences are more significant when we compare the control group with the group of persons with high blood plasma cholesterol levels.

Data on the incidence of coronary artery stenosis are presented in table 5. Since the incidence of stenosis in the different age groups does not differ significantly in cases of sudden cardiac death, the analysis was performed on all cases.

As can be seen from the table, coronary artery stenosis is found in the overwhelming majority of cases of sudden cardiac death. Stenosis is much more frequently seen in cases of sudden death than in the control group; in this group, stenosis of over 50 percent of the lumen of the coronary arteries was observed only in persons over 50 years of age, and the incidence was not over 10-20 percent (10).

In persons with a high level of blood plasma cholesterol, isolated coronary artery stenoses were found less frequently, and multiple stenoses more frequently than in persons with a low level of cholesterol. More severe damage of the coronary arteries in persons with a high blood cholesterol level is also shown by the fact that when they do have stenosis, constriction of the vessel exceeds 50 percent of the lumen, whereas in the group with a low blood

TABLE 5. Incidence of Coronary Artery Stenosis in Sudden Death Cases (in Percent of Number of Observations)

	Plasma-C < 250 mg%	Plasma-C > 250 mg%
Observation group	I	II
Total number of cases with coronary artery stenosis	95.8 ± 2.5	97.9 ± 2.0
Isolated stenosis of one branch of the coronary arteries	25.0 ± 5.3	14.6 ± 5.0
Multiple stenoses of one or several branches of the coronary arteries	70.8 ± 5.6	83.3 ± 5.3
Stenosis of less than 50 percent of the arterial lumen	10.4 ± 3.7*	0.0 ± 0.0
Stenosis greater than 50 percent of the arterial lumen	85.4 ± 4.3*	97.9 ± 2.0

*$p < 0.05$ for comparison of groups I and II. C = Cholesterol.

cholesterol level, stenosis did not exceed 50 percent of the vascular lumen in 10.4 percent of the cases.

In both group I and group II, stenoses were most frequently observed in the left anterior descending coronary artery, and less frequently in the left circumflex coronary artery. We did not note predominant damage of the right coronary artery in any case of sudden death, as similarly reported by Myers and Dewar (11), Baba et al. (12), and Lie and Titus (13).

In addition to studying the degree of atherosclerosis in the aorta, we also determined the cholesterol content. This index was determined separately in macroscopically unchanged sections of the aortic intima, in fatty streaks, and in uncomplicated fibrous plaque. The concentration of cholesterol in the aorta was determined in 53 cases of sudden cardiac death. As a control, we used data obtained from examination of the aorta in essentially healthy persons living in Moscow who had died in accidents (14).

The results of this study showed that the content of cholesterol in macroscopically unchanged sections of the aortic intima did not depend on the blood plasma cholesterol level in persons who died suddenly of CHD (table 6). The lower cholesterol content in the normal intima in the control group resulted from the fact that this group included a larger number of young persons than the group of sudden death victims. According to our observations and information in the literature, the content of cholesterol in the normal intima of the aorta increases with age.

TABLE 6. Cholesterol Content in the Aorta in Cases of Sudden Death and in Essentially Healthy Persons

Group	Cholesterol content, mg/100 mg dry aorta tissue		
	Normal intima (n = 18)	Fatty streaks (n = 16)	Fibrous plaques (n = 19)
I	3.0 ± 0.42*	9.85 ± 1.15**	27.87 ± 5.49
II	2.55 ± 0.26	16.51 ± 2.26	28.2 ± 5.22
III	1.97 ± 0.17	8.18 ± 0.91	29.42 ± 4.20

Group I = Sudden cardiac death, blood plasma cholesterol level below 250 mg%.
Group II = Sudden cardiac death, blood plasma cholesterol level above 250 mg%.
Group III = Essentially healthy persons who died in accidents.
*$p < 0.05$ for normal aortic intima for groups I and III.
**$p < 0.02$ for fatty streaks for groups I and II.

DISCUSSION

Early autopsy of sudden cardiac death victims allowed combined biochemical and morphological study of atherosclerotic changes of the aorta and coronary arteries of the heart and simultaneous study of blood plasma lipids in these persons. This allowed us to analyze and determine the relationship between blood plasma lipoprotein and lipid levels and the degree of atherosclerotic changes in the aorta and coronary arteries in sudden death victims.

The results of such studies may be significant both for evaluating the risk of sudden death as a function of the level of different classes of lipoproteins in the blood, and for refining the significance of lipid metabolism disorders in the pathogenesis of atherosclerosis.

Our studies showed that hypercholesterolemia is not obligatory in cases of CHD. More than half of the sudden death victims were found to have a normal level of cholesterol in their blood plasma. Coronary heart disease is rather more frequently correlated with hypo-α-cholesterolemia. The content of α-cholesterol in the blood plasma was low in 75 percent of the sudden death victims. This, together with an increase in the content of β-cholesterol, leads to an increase in the atherogenic coefficient, that is, the ratio of LDL cholesterol to HDL cholesterol.

The area of lipidosis in the aorta does not correlate with the blood plasma cholesterol level. However, the cholesterol content in the fatty streaks in the aorta is significantly higher in persons with a high level of blood plasma cholesterol. This information is new and worthy of attention.

The area of raised lesions in the aorta is significantly higher in persons with high blood plasma cholesterol level than in persons with a low content of cholesterol in the blood. One factor explaining the correlation between the area of raised lesions in the aorta and the presence of hypercholesterolemia, as well as the correlation between hypercholesterolemia and the cholesterol content in the fatty streaks, may be as follows. The fatty streaks with high cholesterol content (primarily esterified) are organized and transformed to raised lesions more rapidly than the fatty streaks with a lower cholesterol content. The more severe damage found in the coronary arteries of persons with high blood cholesterol levels agrees with this suggestion.

CONCLUSIONS

Hypercholesterolemia was found in 43 percent of persons who had died suddenly of coronary heart disease. In 57 percent of the cases, the blood plasma cholesterol level was normal. A low content of α-cholesterol was found in 75 percent of the sudden death victims. In hypercholesterolemia, there is an increase in the level of β-cholesterol, i.e., the cholesterol of the most atherogenic class of lipoproteins--LDL. In most sudden death victims, HLP of type IIa was found.

The area of lipidosis in the aorta does not correlate with the level of cholesterol in the blood plasma. The content of cholesterol in fatty streaks in the aorta is significantly higher in persons with a high blood plasma cholesterol level.

The area of raised lesions in the aorta of persons with a high blood plasma cholesterol level is significantly greater than in persons with a low blood plasma cholesterol level. The cholesterol content in the raised lesions does not correlate with the blood plasma cholesterol level.

Isolated coronary artery stenoses are observed less frequently, and multiple stenoses are observed more frequently, in persons with a high blood plasma cholesterol level than in persons with a low blood plasma cholesterol level.

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QUANTITATION OF CORONARY ARTERIAL NARROWING AT NECROPSY IN SUDDEN CORONARY DEATH: ANALYSIS OF 31 PATIENTS AND COMPARISON WITH 25 CONTROL SUBJECTS

William C. Roberts, Ancil A. Jones, and Bruce F. Waller

ABSTRACT. A quantitative analysis of the degree and extent of coronary arterial narrowing by atheroslerotic plaques in the entire lengths of each of the four major coronary arteries in a group of patients dying suddenly from coronary heart disease (sudden coronary death) is described at necropsy. A total of 1,564 5 mm long segments of the left main, left anterior descending, left circumflex, and right coronary arteries were examined in 31 patients with sudden coronary death. The observations were compared with findings from examination of 1,100 5 mm segments of the major epicardial coronary artery in 25 control subjects. An average of 25 cm (50 5 mm segments) of coronary artery was examined from each patient and an average of 22 cm (44 5 mm segments) was examined from each control subject. Of the 1,564 5 mm segments examined in the 31 study patients, 557 (36 percent) were 76 to 100 percent narrowed in cross-sectional area by atherosclerotic plaques (compared to 3 percent in the controls); 536 (34 percent) were 51 to 75 percent narrowed (compared to 22 percent in the controls); 360 (23 percent) were 26 to 50 percent narrowed (compared to 42 percent in the controls); and only 111 segments (7 percent) were $\leq$ 25 percent narrowed (compared to 33 percent in the controls). The amount of severe (> 75 percent) narrowing of the right, left anterior descending, and left circumflex coronary arteries was similar; therefore, the concept that "the left anterior descending coronary artery is the artery of sudden death"

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should be discarded. Additionally, the amount of severe (> 75 percent) narrowing in the distal one-half of the right, left anterior descending, and left circumflex coronary arteries was similar to that in the proximal halves of these three arteries. The number of 5 mm coronary segments narrowed 76 to 100 percent in cross-sectional area in the 31 study patients was not affected by the patient's age at death, sex, presence or absence of previous angina pectoris or myocardial infarction, or heart weight.

INTRODUCTION

Although it is recognized that patients dying suddenly from coronary heart disease have considerable narrowing of one or more of the major epicardial coronary arteries (1-9), the exact amount of luminal narrowing in any one or in each of the four major epicardial coronary arteries has not been described. Accordingly, the degree of cross-sectional luminal narrowing in each 5 mm segment of each of the four major epicardial coronary arteries was determined in 31 patients dying suddenly from coronary heart disease and the observations in these patients were compared with 25 control subjects.

PATIENTS STUDIED AND METHODS

Only patients fulfilling the following criteria were included in this study: (a) Death was known to have occurred within 6 hours of the previously witnessed usual state of health. (b) Although the patient may have died in a hospital, at the onset of symptoms, suggestive of myocardial ischemia, the victim was not a patient in a hospital. (c) At least one of the four major (right, left main, left anterior descending, left circumflex) epicardial coronary arteries was > 75 percent narrowed in cross-sectional area at necropsy by atherosclerotic plaque. (d) Ventricular wall, myocardial coagulation necrosis was absent at necropsy. (e) A cause of death, either cardiac or noncardiac, other than coronary luminal narrowing was absent. (f) Chronic congestive cardiac failure had never been present. (g) A cardiac operation had never been performed. On review of clinical and necropsy records in the Pathology Branch of the National Heart, Lung, and Blood Institute (NHLBI), 31 patients fulfilled the above criteria and their hearts and coronary arteries were available for reexamination. Pertinent clinical and noncoronary cardiac necropsy observations in these patients are summarized in table 1.

Control subjects, similar in age and sex to the study patients, were selected on the basis of the following criteria: (a) Death occurred from a noncardiac condition. (b) Symptoms suggesting or indicating myocardial ischemia or cardiac dysfunction during life were absent. (c) Systemic hypertension (> 140 mm Hg systolic and/or > 90 mm Hg diastolic) was absent. (d) Therapeutic mediastinal irradiation was absent. (e) Cardiomegaly (> 400 g for men and > 350 g for women) was absent at necropsy. A total of 25 subjects who fulfilled the above criteria were selected as controls: 13 died

TABLE 1. Quantitation of Coronary Narrowing in Sudden Coronary Death (SCD) Patients and in Control (C) Subjects: Clinical Observations and Noncoronary Cardiac Findings

Group	No. Pts.	Age (yrs) Range (mean)	Sex		Historical Data					Laboratory Data						Autopsy Data		Gross LV Scars	
			M	F	AP	Past AMI	Chronic CHF	DM	SH	C done	C >200	T done	T >200	C Values Range (mean)	T Values Range (mean)	Heart Weight Gm Range (mean)	No. Pts. Increased Heart Weight[a]	Wall Thickness < ½	Wall Thickness > ½
SCD	31	22 - 85 (47)	24 (77%)	7 (23%)	16/29 (55%)	0/31 (0%)	0/31 (0%)	6/29 (21%)	5/19 (26%)	10/31	9*/10	9/31	4/9	198 - 700 (361)*	86 - 1000 (362)	260 - 540 (420)*	21/31 (68%)	6 (19%)	4[b] (13%)
C	25	25 - 74 (49)	18 (72%)	7 (28%)	0/25	0/25	0/25	0/25	0/25	21/25	5**/21	4/25	1/4	128 - 245 (176)**	106 - 202 (151)	200 - 400 (288)**	0/25	0	0

Abbreviations: AMI = Acute myocardial infarct (which healed); AP = angina pectoris; C = serum cholesterol level (in mg per dl); CHF = congestive heart failure; DM = diabetes mellitus; LV = left ventricle; SH = systemic hypertension; T = serum triglyceride level (in mg per dl)

* – ** $p < 0.05$

[a] Weight > 400 gms in men and > 350 gms in women.

[b] Posterior wall scars in each. These patients never had evidence clinically of AMI.

of carcinoma or sarcoma; 5 died of leukemia; 4 died of lymphoma; and 1 each died of heat stroke, gunshot wound, and acute infection.

The coronary arteries in all patients and in control subjects were studied in similar fashion (figure 1). The hearts were fixed for at least 1 day in formalin. The four major epicardial coronary arteries then were excised intact, X-rayed, and fixed for at least 1 other day. Following decalcification (if necessary), each of the four major coronary arteries were cut transversely to their longitudinal axes into approximately 5 mm long segments and each segment was labeled sequentially from either its aortic ostium or from its origin from the left main. The number of 5 mm segments examined in the study patients and control subjects is summarized in table 2.

The 5 mm segments were labeled, dehydrated (with alcohol and xylene), embedded in paraffin, and two histologic sections were cut and stained from each paraffin block. The Movat stain was used on one histologic section and all determinations of luminal narrowing were based on examination of the Movat stained sections. The degrees of narrowing were based on histologic examination of each cross section magnified 25 to 50 times. Judgment regarding the degree of luminal narrowing of each 5 mm segment was based on the degree of luminal obliteration within the luminal circle bordered by the internal elastic membrane. The circle was visually subdivided into four equal-sized quadrants, and the percentage of cross-sectional area luminal narrowing in each 5 mm segment was determined as follows: 0-25, 26-50, 51-75, and 76-100.

In addition to sectioning the major epicardial coronary arteries, at least three histologic sections extending from the endocardium to the epicardium and for at least 2 cm in circumferential dimension were prepared from left ventricular myocardium from each patient and stained by hematoxylin and

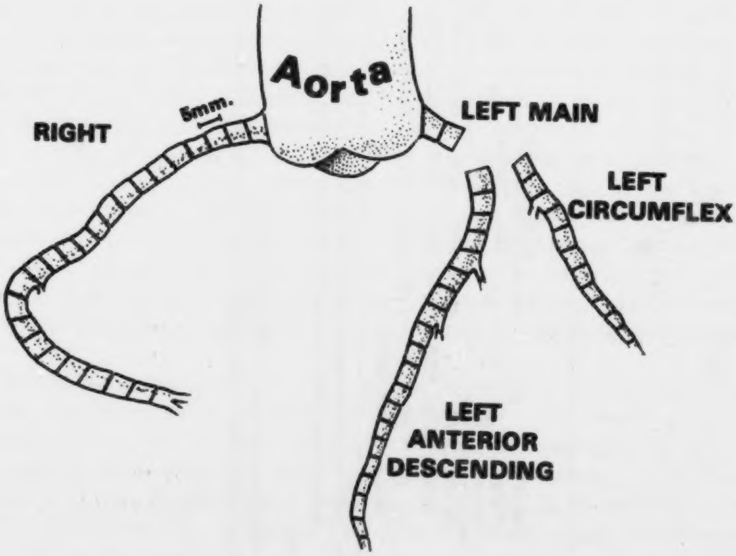

FIGURE 1. Diagram of the four major coronary arteries, each divided into 5 mm segments, as was done in each of the study patients and in the control subjects.

eosin. Gross myocardial fibrosis (table 1) (10 patients) and the absence of myocardial coagulation necrosis were confirmed in each patient by histologic examination.

RESULTS

Among the 31 patients with sudden coronary death, a total of 121 major coronary arteries were examined (the left main was not examined in 3 patients). Among the 25 control subjects, a total of 96 major coronary arteries were examined (the left main was not examined in 4 subjects). The maximal degree of cross-sectional area luminal narrowing by atherosclerotic plaque in one or more of the four major coronary arteries in each of the 31 study patients and in each of the 25 control subjects is summarized in table 3. All study patients had 76 to 100 percent cross-sectional area luminal narrowing by atherosclerotic plaque in one or more of their four major coronary arteries, and 7 (28 percent) of the 25 control subjects also had this degree of narrowing.

The number of arteries narrowed 76 to 100 percent, however, between the study patients and control subjects was quite different. Of the 31 study patients, 29 (94 percent) had two or more of their four major coronary arteries narrowed > 75 percent in cross-sectional area by atherosclerotic plaque, whereas only 3 (12 percent) of the 25 control subjects had two or more arteries narrowed to this degree. Of the study patients, 3 had > 75 percent narrowing by atherosclerotic plaque of all four major coronary arteries; 20 patients had three coronary arteries narrowed to this degree; 6 patients had two arteries narrowed to this degree; and 2 patients had a single artery narrowed to this extent. Thus, of the possible 124 major coronary arteries in the 31 study patients (actually only 121 arteries were examined), 86 (67 percent) were > 75 percent narrowed in cross-sectional area by atherosclerotic plaque for an average of 2.8/4.0 coronary arteries per study patient. If the left main coronary artery was excluded, 83 (89 percent) of the other 93 major (right, left anterior descending, and left circumflex) coronary arteries were narrowed > 75 percent in cross-sectional area by atherosclerotic plaque, for an average of 2.7/3.0 coronary arteries per study patient.

Of the seven control subjects with > 75 percent cross-sectional area narrowing by atherosclerotic plaque, all four major coronary arteries were narrowed to this degree in one subject, two arteries were similarly narrowed in three subjects, and only one artery was narrowed to this extent in three subjects. Thus, of the possible 100 major coronary arteries in the 25 control subjects (actually only 96 arteries were examined), a total of 13 (13 percent) were narrowed > 75 percent in cross-sectional area by atherosclerotic plaque for an average of 0.5/4.0 coronary arteries per control subject. If the left main coronary artery was excluded, 12 (16 percent) of the 75 other major (right, left anterior descending, left circumflex) coronary arteries were narrowed > 75 percent in cross-sectional area by atherosclerotic plaque for an average of 0.5/3.0 coronary arteries per control subject.

TABLE 2. Number of 5 mm Long Segments of Major Epicardial Coronary Artery Examined Per Patient: Sudden Coronary Death (SCD) (31 Patients) Versus Controls (25 Subjects)

Coronary Artery	Number (Range [mean]) of 5 mm Segments Per Patient	
	SCD (31 patients)	C (25 subjects)
Right	8 - 31 (20.0)	12 - 27 (18.1)
Left main	1 - 4 (1.8)	1 - 3 (1.6)
Left anterior descending	13 - 27 (18.8)	11 - 26 (15.3)
Left circumflex	4 - 20 (10.1)	3 - 27 (9.3)
Total Segments of 4 Coronary Arteries Per Patient	39 - 78 (50.5)	31 - 67 (44.0)

TABLE 3. Number of 31 Patients With Sudden Coronary Death (SCD) And 25 Control Subjects (C) Showing Maximal Luminal Narrowing of One or More Epicardial Coronary Arteries by Atherosclerotic Plaque

Artery	Percent Cross-Sectional Area Luminal Narrowing								Total	
	0 - 25		26 - 50		51 - 75		76 - 100			
	SCD	C	SCD	C	SCD	C	SCD	C	SCD	C
1. R	–	–	0	0	0	3	0	1	0	4
2. LAD	–	–	0	0	0	1	2	2	2	3
3. R, LAD	–	–	0	1	0	4	3	2	3	7
4. R, LC	–	–	0	0	0	0	3	0	3	0
5. LAD, LC	–	–	0	0	0	2	0	1	0	3
6. R, LAD, LC	–	–	0	2	0	3	20	0	20	5
7. R, LM*, LAD, LC	0	1	0	1	0	0	3	1	3	3
Total (%)	0 (0)	1 (4)	0 (0)	4 (16)	0 (0)	13 (52)	31 (100)	7 (28)	31 (100)	25 (100)

* Sections of LM not examined in 3 SCD patients and in 4 control subjects.

Abbreviations: R = Right coronary artery; LM = Left main coronary artery; LAD = Left anterior descending coronary artery; LC = Left circumflex coronary artery.

Two of the 31 patients had thrombi in one coronary artery. In each patient the thrombus was superimposed on atherosclerotic plaque and the amount of luminal obliteration contributed entirely by the thrombus was not included in the calculations of luminal narrowing, only that part resulting from atherosclerotic plaque. None of the control subjects had a coronary thrombus.

The results of quantitative analysis of the 5 mm long coronary segments in both study patients and control subjects are summarized in table 4. Of the 1,564 5 mm long segments of major coronary arteries examined in the 31 study patients, 557 segments (36 percent) were 76 to 100 percent narrowed in cross-sectional area by atherosclerotic plaque (compared to 3 percent in the controls); 536 (34 percent) were 51 to 75 percent narrowed (compared to 22 percent in the controls); 360 (23 percent) were 26 to 50 percent narrowed (compared to 42 percent in the controls); and 111 (7 percent) were 0 to 25 percent narrowed (compared to 33 percent in the controls). The mean percent of 5 mm coronary segments narrowed 0-25, 26-50, 51-75, and 76-100 percent was significantly ($p < 0.05$) different between study patients and control subjects at each of the four levels of narrowing (figure 2).

TABLE 4. Number and Percent of 5 mm Long Segments of the Four Major Epicardial Coronary Arteries in 31 Patients With Sudden Coronary Death (SCD) and in 25 Control Subjects (C) Showing the Four Grades of Cross-Sectional Luminal Narrowing

Coronary Artery		Percent Cross-Sectional Area Luminal Narrowing								Total	
		0 - 25		26 - 50		51 - 75		76 - 100			
		SCD	C	SCD	C	SCD	C	SCD	C	SCD	C
LM*	No.	11	8	19	20	13	3	5	2	48	33
	%	(22)	(24)	(40)	(61)	(28)	(9)	(10)	(6)	(100)	(100)
LAD	No.	55	121	143	154	181	85	205	22	584	382
	%	(9)	(32)	(24)	(40)	(31)	(22)	(36)	(6)	(100)	(100)
LC	No.	20	90	74	96	104	43	115	3	313	232
	%	(6)	(39)	(24)	(44)	(33)	(16)	(37)	(<1)	(100)	(100)
R	No.	25	144	124	200	238	106	232	3	619	453
	%	(4)	(32)	(20)	(44)	(38)	(23)	(38)	(<1)	(100)	(100)
Totals	No.	111	363	360	470	536	237	557	30	1564	1100
	%	(7)	(33)	(23)	(42)	(34)	(22)	(36)	(3)	(100)	(100)

*Sections not examined in 3 SCD patients and in 4 control subjects.

Abbreviations: LM = Left main coronary artery; LAD = Left anterior descending coronary artery; LC = Left circumflex coronary artery; R = Right coronary artery.

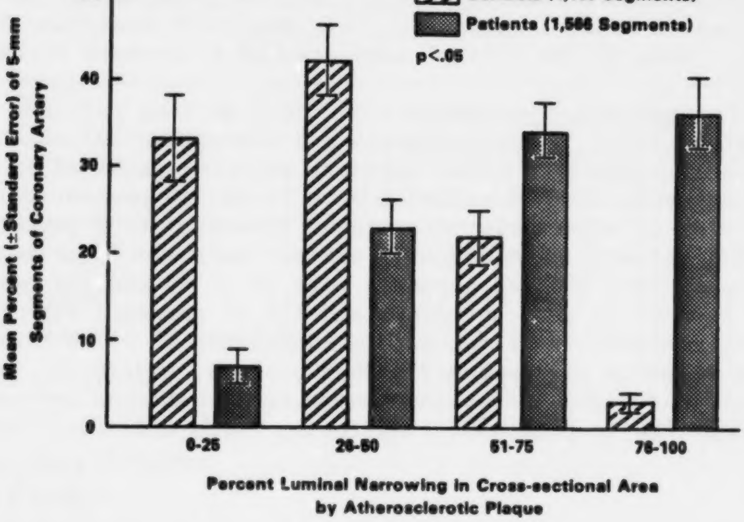

FIGURE 2. Percent of 5 mm segments of all four major coronary arteries narrowed to various degrees in the 31 study patients and in the 25 control subjects.

The number and/or percent of 5 mm long segments narrowed to various degrees in each of the four major coronary arteries also is summarized in table 4 and in figures 3 and 4. The mean percent of 5 mm segments of left anterior descending, left circumflex, and right coronary arteries narrowed 0-25, 26-50, 51-75, and 76-100 percent was significantly ($p < 0.05$) different between study patients and control subjects at each of the four degrees of narrowing (figures 3 and 4). No significant differences were detected in the mean percent of segments of the left main coronary artery narrowed 0-25, 26-50, 51-75, and 76-100 percent between study patients and control subjects (figures 3 and 4). The mean percent of 5 mm segments of the right, left anterior descending, and left circumflex coronary arteries at each of the four degrees of narrowing was similar in the study patients (figures 3 and 4). Likewise, the mean percent of segments of each of these three major coronary arteries narrowed to varying degrees in the control subjects was similar (figure 3). The degree of narrowing of the left main coronary artery in both study patients and control subjects was quite different from the mean percent of narrowing of the segments in the other three major coronary arteries.

The mean percent of 5 mm segments narrowed 76 to 100 percent in cross-sectional area in the proximal one-half of the right, left anterior descending, and left circumflex coronary arteries was similar to the mean percent of 5 mm

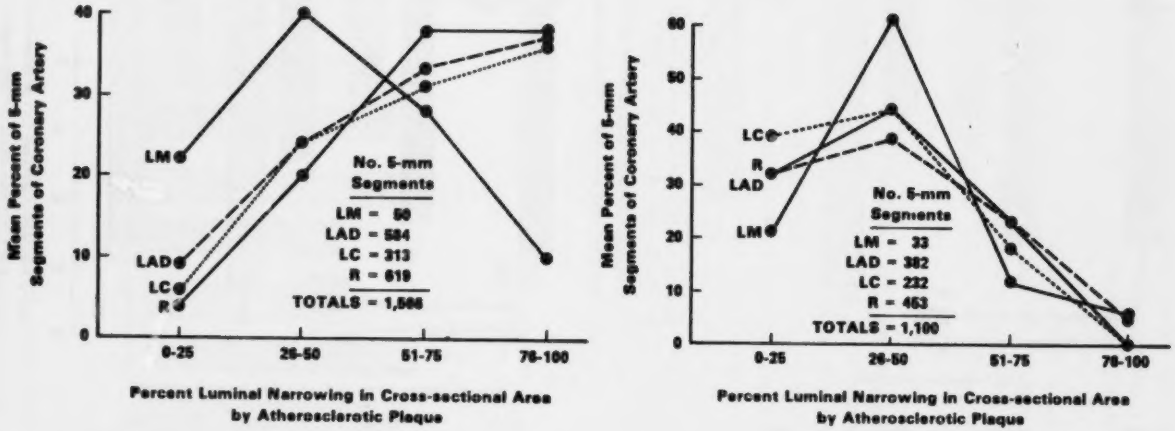

FIGURE 3. Mean percent of 5 mm segments of each of the four major coronary arteries narrowed to various degrees in the 31 patients who died from sudden coronary death (left) and in the 25 control subjects (right). The amount of luminal narrowing of the right (R), left anterior descending (LAD), and left circumflex (LC) coronary arteries is similar. The degree of severe narrowing of the left main (LM) coronary artery is considerably less than that of the other three arteries in the study patients.

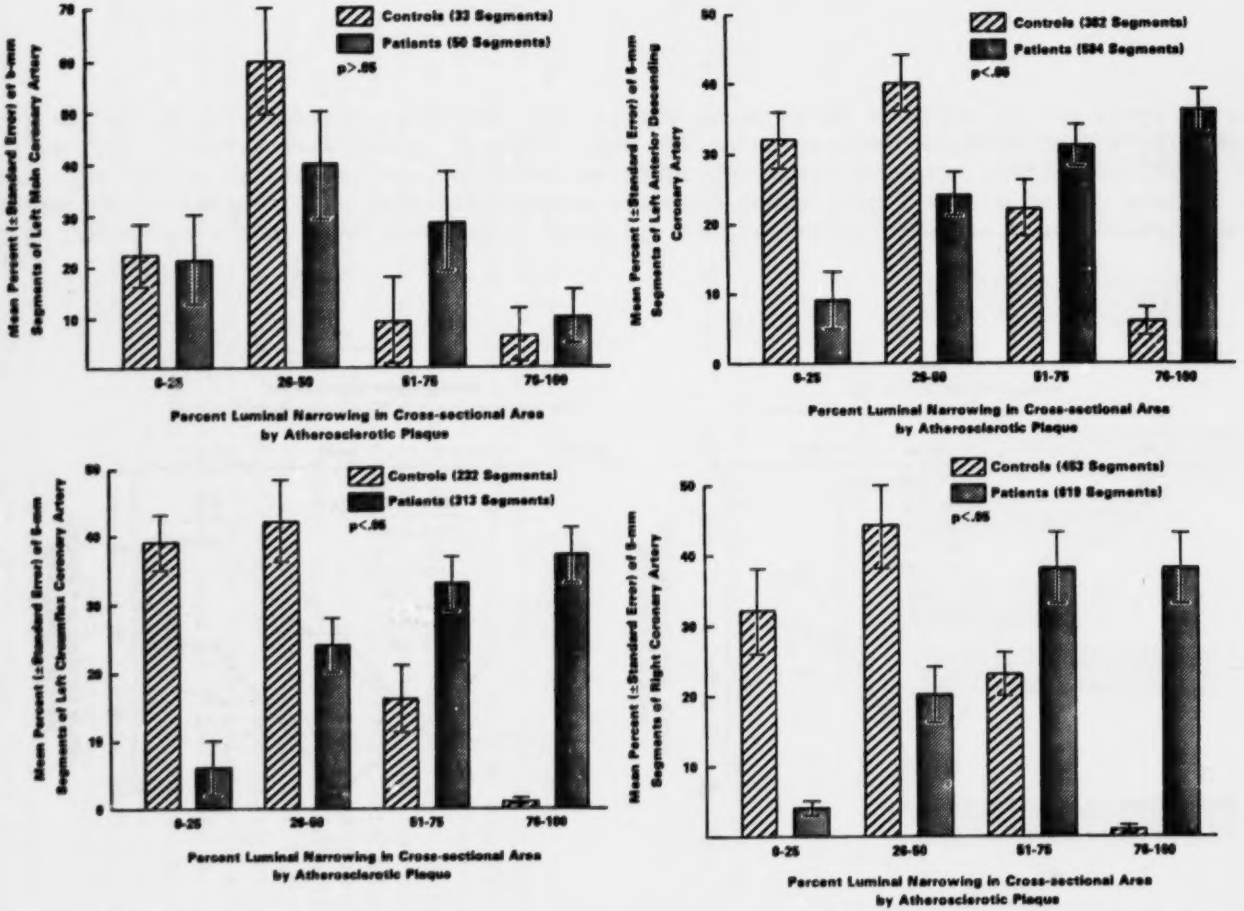

FIGURE 4. Mean percent of 5 mm segments of each of the four major coronary arteries narrowed to various degrees in the 31 patients who died from sudden coronary death and in the 25 control subjects. Upper left: Left main coronary artery. Upper right: Right coronary artery. Lower left: Left anterior descending coronary artery. Lower right: Left circumflex coronary artery.

segments similarly narrowed in the distal one-half of these arteries (figure 5). The mean percent of segments narrowed > 75 percent in the proximal one-half of these three arteries was 39 percent, and in the distal one-half, the mean percent was 34 ($p > 0.05$). The distal half of the left anterior descending tended to have a lower percentage of segments > 75 percent narrowed than did the proximal half of this artery, but the opposite was true for both the right and left circumflex coronary arteries. The percent of 5 mm segments narrowed > 75 percent in cross-sectional area by atherosclerotic plaque in the first 2 cm of left anterior descending coronary artery also tended to be higher than the percent narrowed > 75 percent in the remainder of this artery (43 ± 7 percent versus 33 ± 30 percent) ($p > 0.05$). The opposite occurred with the left circumflex coronary artery. The mean percent of 5 mm segments narrowed 76 to 100 percent in cross-sectional area by atherosclerotic plaque in its first 2 cm tended to be less than the mean percent of segments narrowed to this degree distal to that point (32 ± 5 percent versus 38 ± 6 percent) ($p > 0.05$).

The relationship of five clinical or morphological parameters in the study patients to the mean percent of 5 mm coronary segments narrowed > 75 percent in cross-sectional area is summarized in table 5. Comparison of patients in three different age groups (< 45, 45-65, and > 65 years) showed no significant differences in mean percent of coronary segments narrowed > 75 percent. Likewise, no significant differences in mean percent of coronary segments narrowed > 75 percent in the study patients were apparent when comparing men to women, when comparing those with and those without previous angina pectoris, when comparing those with increased and those with normal heart weights, and when comparing those with and those without healed, transmural left ventricular myocardial infarcts (table 5).

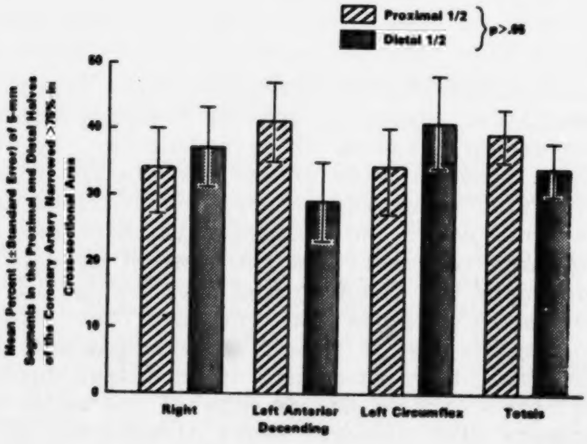

FIGURE 5. Mean percent of 5 mm segments of the right, left anterior descending, and left circumflex coronary arteries narrowed greater than 75 percent in cross-sectional area in the proximal one-half compared to the distal one-half of each of the three arteries. The mean percent of 5 mm segments severely narrowed was similar in both proximal and distal halves of these three arteries.

TABLE 5. Mean Percent of Total 5 mm Segments in the Four Major (Right, Left Main, Left Anterior Descending, and Left Circumflex) Epicardial Coronary Arteries Narrowed > 75 Percent in Cross-Sectional Area by Atherosclerotic Plaque in 31 Patients With Sudden Coronary Death (SCD): Comparison of Five Clinical or Morphological Parameters

Parameter Analyzed	No. Pts.	Mean Percent (± Standard Error) of 5 mm Coronary Segments Narrowed >75% in Cross-Sectional Area by Atherosclerotic Plaque	p Value
Age (Years)			
< 45	15	36 ± 6	> .05
45 - 65	13	33 ± 5	
> 65	3	36 ± 8	
Sex			
Men	24	36 ± 4	> .05
Women	7	30 ± 8	
Angina Pectoris			
Present	18	34 ± 8	> .05
Absent	13	35 ± 5	
Heart Weight			
Increased*	21	36 ± 5	> .05
Normal	10	31 ± 5	
Healed Transmural Infarct, Left Ventricle			
Present	4	51 ± 4	> .05
Absent	27	32 ± 4	

* > 400 gm in men; > 350 gm in women.

DISCUSSION

In many cardiovascular diseases, quantitation of the degree of severity of the condition is commonplace. Examples are the everyday recording of exact pressure gradients across stenotic valves or determination, by several means, of the magnitude of a shunt from one side of the circulation to another. Angiography, and more recently, echocardiography, has provided means of measuring cavity size and the latter can measure wall thickness as well. Recent attempts have been made to quantitate the size of myocardial infarcts. For years, coronary angiography has demonstrated "narrowing" in one or more epicardial coronary arteries in patients with coronary heart disease but the exact degree of narrowing in any one or in all major epicardial coronary arteries has not been precise. Many thousands of autopsies during this century have demonstrated severe narrowings in one or more of the major epicardial coronary arteries of patients with coronary heart disease but no attempts have been made to quantitate the degree and extent of coronary luminal narrowing at necropsy in these patients.

The present study was undertaken to provide quantitative information on the degree and extent of luminal narrowing in each of the four major epicardial coronary arteries in patients dying suddenly from coronary heart disease. Over 25 cm (50 5 mm segments) of the four major (right, left main, left anterior descending, and left circumflex) epicardial coronary arteries were examined in each of 31 patients, or a total of 790.5 cm of major coronary artery. Each 1 cm segment was divided into equal-sized halves and a histologic section was examined from each 5 mm segment. A total of 1,564 5 mm segments of coronary artery, therefore, was examined from the entire group of 31 patients. The percent of cross-sectional area narrowing by atherosclerotic plaques in each 5 mm segment was graded as follows: 0-25, 26-50, 51-75, and 76-100 percent. Of the 31 patients studied, in over one-third (36 percent) of the entire lengths of the four major epicardial coronary arteries studied, the lumens were > 75 percent narrowed in cross-sectional area by atherosclerotic plaque. (It is worth recalling that a 75 percent cross-sectional area narrowing is equivalent to a 50 percent diameter reduction on the luminogram) (10). In addition, another 34 percent of the major coronary arteries (average length = 25.5 cm) had luminal narrowings between 51 and 75 percent in cross-sectional area. Thus, of the 31 patients with sudden coronary death, 70 percent of the lengths of the four major epicardial coronary arteries were > 50 percent narrowed in cross-sectional area by atherosclerotic plaque. Only 3 percent of the lengths of the four major coronary arteries in the control subjects were narrowed > 75 percent in cross-sectional area by atherosclerotic plaque in contrast to 36 percent of the lengths of the four major coronary arteries in the study patients narrowed to this degree.

Not only was severe narrowing widespread in the study patients, but some degree of narrowing was present in virtually every 5 mm segment of coronary artery studied. As shown in table 4 and figure 2, 93 percent of the coronary tree studied was over 25 percent narrowed in cross-sectional area, or only 7 percent of the coronary tree was ≤ 25 percent narrowed in cross-sectional area, and not a single 5 mm segment was entirely normal in a single patient. In contrast, one-third of the coronary tree in the 25 control subjects was ≤ 25 percent narrowed in cross-sectional area by atherosclerotic plaque. Thus, coronary atherosclerosis among patients with sudden coronary death is a diffuse process, involving, for practical purposes, all segments of all four major epicardial coronary arteries.

Surprisingly, the degree of severe narrowing in the distal halves of the right, left anterior descending, and left circumflex coronary arteries in the study patients (and also in the controls) was just as great as in the proximal halves of these arteries. The mean percent of 5 mm segments narrowed > 75 percent in cross-sectional area by atherosclerotic plaque in the proximal one-half of each of these three coronary arteries was similar to the mean percent of 5 mm segments similarly narrowed in the distal one-half of these arteries (figure 6). The implications of this observation for aortocoronary bypass grafting are obvious, but it has already been demonstrated that most coronary anastomoses are at sites of atherosclerotic plaquing (11) and, nevertheless, myocardial perfusion apparently is rarely not increased.

Although the left anterior descending coronary artery has been called "the artery of sudden death," this study shows that there is no justification for that belief. As shown in figures 3 and 4, the degree of narrowing of the left anterior descending, left circumflex, and right coronary arteries was similar; that is, the percent of 5 mm coronary segments narrowed 0-25, 26-50, 51-75, and 76-100 in each of these three arteries was similar. The left main coronary artery was considerably less narrowed than the other three major coronary arteries.

The amount of severe (> 75 percent) coronary narrowing present in the patients with sudden coronary death could not be correlated with the patients' age, sex, presence or absence of previous angina pectoris, heart weight at necropsy, or the presence of transmural left ventricular scars at necropsy (table 5). Thus, the number of segments of coronary artery narrowed > 75 percent in cross-sectional area by atherosclerotic plaque among patients with sudden coronary death was similar in the young and old patients, in both men and women, in those with and without previous episodes of angina pectoris or myocardial infarction, and in those with either normal sized or enlarged hearts.

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NEUROHUMORAL DISTURBANCES IN THE HEART AND ITS INTRA- AND EXTRAORGAN NERVE SYSTEM IN SUDDEN DEATH COMPARED WITH THE AMOUNT OF NERVOUS SYSTEM MEDIATORS IN THESE REGIONS AS A FUNCTION OF AGE

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ABSTRACT. Quantitative indices for a focal lesion of the myocardial adrenergic nerve plexuses, with relative preservation of the cholinergic plexuses, were determined in 80 cases of sudden cardiac death (SCD) in persons 25-60 years of age. The content of mediators in the adrenergic nerve plexuses is significantly lower than normal, although after age 40 a reduction in the catecholamine content in the myocardial nerve plexuses is usually observed. An increase in sensitivity to catecholamines was apparent in isolated myocardial striae taken from hearts after SCD. The nonuniform, mosaic nature of the reduction in catecholamine content in the adrenergic plexuses of the heart matched the results of biochemical studies of epinephrine, norepinephrine, and DOPA. Focal lesions of the microcirculatory bed were also found in cases of SCD; these were determined during reaction to pseudocholinesterase and with the electron microscope. Degenerative impairment of the neurons of the sympathetic ganglia was established in studying the extraorgan parts of cardiac innervation. Nothing in the way of definite impairment was detected in the nuclei of the vagus nerves in the medulla oblongata. Dyscirculatory and dystonic changes in the hypothalamus, with conductor zone edema, were revealed, as was inhibition of the hypothalamohypophysoadrenal system. Hormone-mediator dissociation in the hypothalamus, with an increase in the quantity of epinephrine and a reduction in norepinephrine and DOPA, was established biochemically. Neurohumoral disturbances were established as the deep precursors of SCD. Preventive examination of the condition of the autonomic nervous

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system of persons with heart rhythm impairments, particularly after age 35-40, is recommended.

INTRODUCTION

In recent years, the principles of nervosism have been revived and refined based on a new methodology in the cardiology clinic. Representations of the neurogenic nature of the most important cardiovascular diseases in the Soviet Union are based on the classic works of Sechenov (1), Pavlov (2), and their successors. These concepts were further developed in cardiology as part of the work of Lang (3) and Myasnikov (4), and their schools. Today, scientists in the United States are looking at neurogenic factors in the genesis of the most important cardiovascular diseases (5-8).

Changes in the cardiac nerve apparatus in sudden cardiac death were demonstrated in the report by Shvalev, Stropus, and Morozov (9) at the USA-USSR First Joint Symposium on Sudden Death, held in Yalta in 1977. In this report, particular attention was devoted to the damage to this apparatus by adrenergic (adaptive-trophic) efferent innervation of the myocardium and blood vessels. Mostly reactive changes in the intracardiac cholinergic neurons were also established, as well as the reactive-degenerative damage to the heart receptors and in the sinocarotid reflexogenic zone preceding sudden death.

A more in-depth study of innervation and microcirculation in the heart in SCD has been conducted over the past 2 years. The data obtained from this study were compared with the morphobiochemical features of the autonomic centers of the brain (the hypothalamus and medulla oblongata) that coordinate myocardial adrenergic and cholinergic innervation, and with the condition of the extracardiac receptor zones that participate in regulating heart function.

MATERIALS AND METHODS

Material from 80 males and females from 25 to 60 years of age who were autopsied 2-5 hours after death were used in this study of SCD. In order to study normal values as a function of age, we used 19 series of human embryos 5.5 to 12 weeks old, 63 fetuses in various stages of development, and 15 infants. Material from 38 victims of road accidents was used to quantitatively study nerve plexuses in the postnatal period. Adrenergic nerve elements were determined by a modification of Falck's method, and by incubation of sections in a 2 percent solution of glyoxalic acid. Cholinergic nerve elements were determined according to Karnovsky and Roots (10). The series of embryos were impregnated with silver salts. Monoamine oxidase (MAO) activity was determined according to Glenner et al. (11). The density of the nerve plexuses was calculated by the point method (12). Morphometry of the preparations was performed in collaboration with Bykov and Tsinadze using an IGPI-4 television microscope. A color television analyzer (TATsI-3) was also used in collaboration with Fedchenko.

Biochemical determination of norepinephrine, epinephrine, and their precursor, DOPA, was made in nine different zones of the myocardium in 44 cases of SCD. The Matlina and Rakhmanova trioxyindole method (13) was used. The hypothalamus was studied in the form of paraffin sections stained by Nissl's method using cresyl violet and toluidine blue at pH 5.6, Schiff's reagent, hematoxylin-eosin and paraldehyde, and fuchsin as per Homori. Epinephrine, norepinephrine, and DOPA were determined biochemically in 31 cases using parallel pieces of the hypothalamus and the same trioxyindole method.

RESULTS

Previous work by Shvalev et al. (14) was the premise for research on the age norm. This work established two stages in the prenatal development of the autonomic nerve plexuses in mammals and man: premediator and mediator (figure 1). In addition to earlier data obtained on the beginning of the mediator stage in the human embryo at 8-10 weeks of prenatal life, when activity of the mediators appears for the first time in the cholinergic and then in the adrenergic nerve plexuses of the large vessels and the heart, recent findings have shown damage to the dynamics of this process. In approximately 10

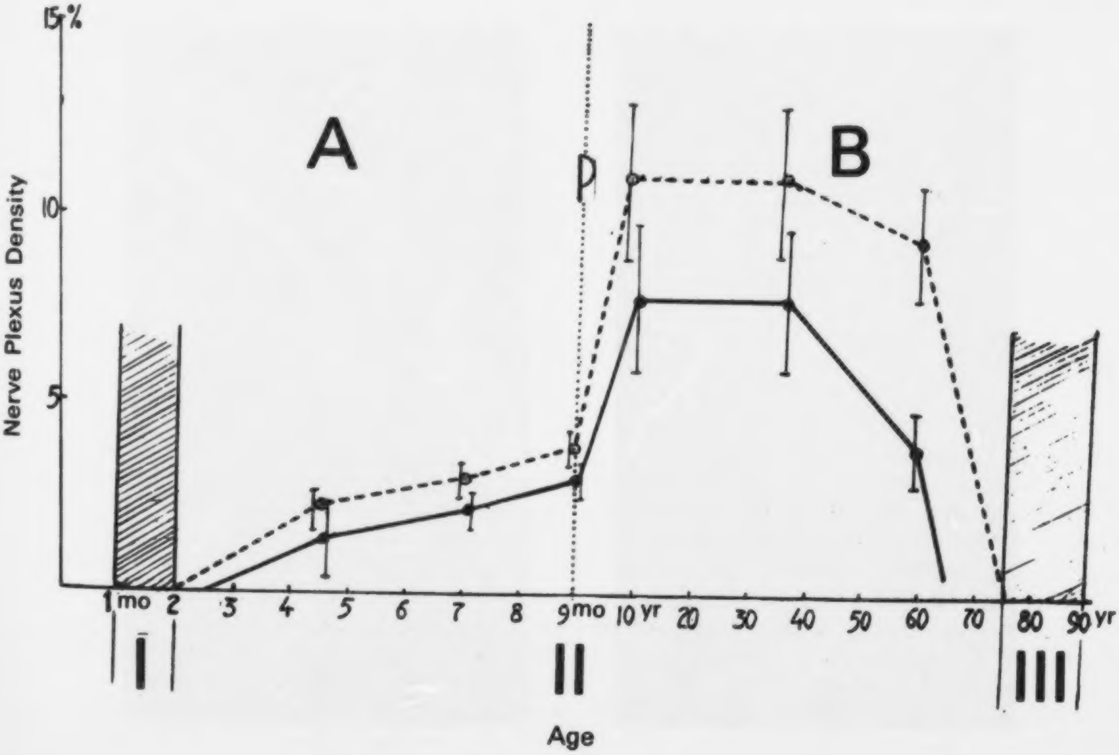

FIGURE 1. Development and involution, by age, of the cholinergic (⊙---⊙) and adrenergic (●——●) intracardiac nerve plexuses in the normal human heart. A = Prenatal period. B = Postnatal period. P = Moment of birth. I = Premediator stage of the ontogenesis of the autonomic nervous plexuses of the cardiovascular system. II = Mediator stage. III = Postmediator stage.

percent of the cases, there is a delay in the beginning of the mediator stage of development of nerve plexuses in the heart. Impairment in the formation of the heart regulator adaptative-trophic nerve connection can be caused by genetic factors, as well as by the effect of drugs, alcohol, and nicotine on pregnant women (15). Impairment in the development of cardiac nerve plexuses, and of other organs, can be combined with anomalies in the histogenesis of their tissue components. This establishes the prerequisites for postnatal changes in the internal organs.

An increase in the complexity of the cholinergic and adrenergic plexuses in the heart of a normal human fetus can be marked at the middle of prenatal life, at the seventh month, and at the time of birth (figure 2).

We noted a lag in the differentiation of the adrenergic nerve plexuses in the hearts of 4 of the 15 infants examined. It is normal to see a further maturation of the mediator systems in the heart after birth. The density of the auricular cholinergic nerve plexuses is 9-13 percent by the seventh year, and the density of the adrenergic nerve plexuses is 7-11 percent. It has been

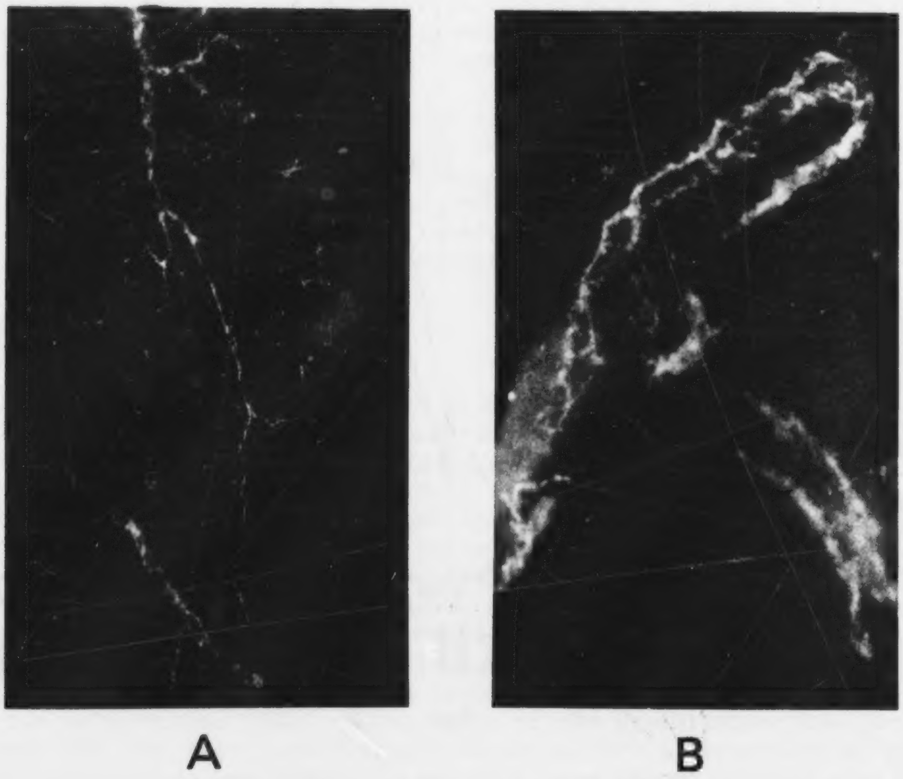

FIGURE 2. Sections of forming adrenergic nerve plexuses in the heart of a human 9-month-old fetus (right atria). A = Plexus around a branch of the coronary artery. B = Subepicardial plexus. Incubation in 2 percent glyoxalic acid. Field 40. Stain Gomal 3.

established that these indices retain their constancy until approximately age 40, after which the density of the adrenergic plexuses in the heart drops to 3-5 percent by age 60, whereas the concentration of cholinergic plexuses drops more slowly (to 8-11 percent). Initially, catecholamine can no longer be distinguished in the adrenergic plexuses in old age and senility, after which the activity of acetylcholinesterase in the cardiac perivascular nerve plexuses disappears. The postmediator stage of ontogenesis of the autonomic plexuses begins.

Development of the sympathoadrenal system is thus nonuniform in ontogenesis. Determination by Kiseleva of the activity of this system as a function of the excretion of catecholamines reflects the age shifts in the condition of the system: a gradual increase in activity as a result of the formation of chromaffin and sympathetic parts, an increase in functional reserves, and a gradual reduction in activity with involution of the nervous system (figure 3). It should be pointed out that the graph of the ontogenesis of the mediators of the intracardiac nerve plexuses coincides rather well with data from the biochemical analysis of catecholamine excretion by healthy persons at various ages. According to our data, the activity of the sympathoadrenal system gradually increases reaching a maximum at ages 12-16 to 21-35; the process then reverses. The functional reserves of the sympathoadrenal system by biochemical determination increase in the 16- to 30-year-old period, reaching a maximum at age 31 to 40.

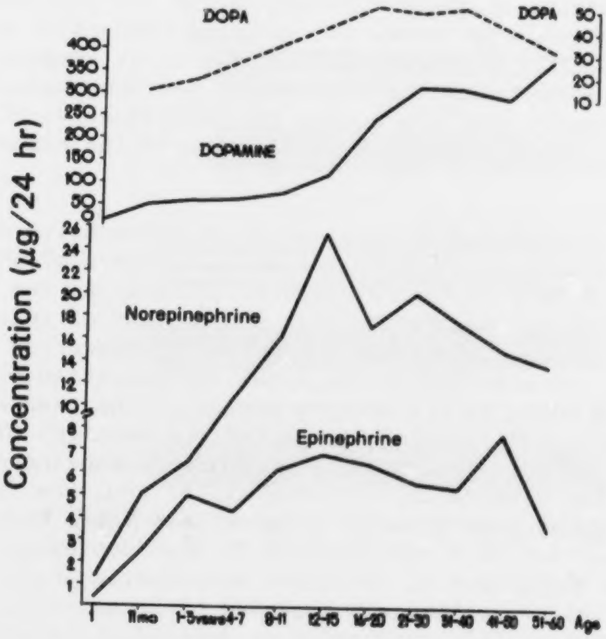

FIGURE 3. Excretion of catecholamines and DOPA in urine by healthy persons.

Abundant cholinergic and adrenergic nerve plexuses are normal in the adult male heart. Numerous nerve ganglia predominate in the atrial epicardium. The most abundant reception fields of vagal and spinal origin are combined with the efferent plexuses in the myocardium and under the epicardium. The adrenergic plexuses are proliferated perivascularly and along the conduction system, and are in direct proximity with the cardiomyocytes in the myocardium. If the adrenergic plexuses are proliferated in all parts of the heart, mostly in the right half, then the cholinergic plexuses are primarily concentrated in the walls of the atria and along the conduction system, and to a much lesser extent, in the heart ventricles.

Changes in the Intracardiac Nerve Plexuses and Microcirculatory Bed of the Myocardium in SCD

In-depth study of the phenomenon of deep focal depletion of catecholamines in the adrenergic nerve plexuses, reported at the USA-USSR First Joint Symposium on Sudden Death, has now made it possible to obtain definitive quantitative data (figure 4). The density of the distribution of adrenergic fibers in all parts of the heart in SCD is slight, according to the data of Stropus and Zhuchkova. In addition to areas of complete fallout of luminescence from adrenergic fibers, there are parts with terminals in the form of a little chain of disconnected varicosities (figure 5). The density of adrenergic nerve plexuses, according to the mean data for SCD, was from 0.5 percent to 1.5 percent in the right atrium, from 0.18 percent to 0.4 percent in the left atrium, from 0.11 percent to 0.4 percent in the right ventricle, and ordinarily only traces of adrenergic fibers with a mean density of about 0.04 percent in the left ventricle. There were few branching luminescent fibers in the heart conduction system. Changes in the intracardiac neurons were described at the first symposium in Yalta in 1977. We later established that the cholinergic neurons characteristic of the heart develop a pronounced reaction during the determination of monoaminooxidase, particularly in the microganglia in the area of the sinus node of the conduction system.

Kiseleva made a biochemical determination of epinephrine and DOPA in parallel with a histochemical study of the heart in cases of SCD and showed that, in SCD without recent foci of myocardial infarction, the content of norepinephrine in the heart is greater than the content of epinephrine. This relationship was the reverse if a recent myocardial infarction was established in the cases of SCD. Interestingly, the total concentration of catecholamines in the myocardium was lower in the absence of infarction than when infarction was present (table 1). DOPA content in the various sections of the heart was not uniform. This content exceeded the sum of norepinephrine and epinephrine in most sections of the myocardium, but only traces could be found in individual areas when there had been a recent infarction. These biochemical studies indicate heavy consumption of norepinephrine in the development of myocardial infarction against a background of increased sympathetic activity. A considerable reduction in catecholamines was observed histochemically in the cardiac nerve plexuses in cases of SCD accompanied by myocardial infarction.

In her study of the heart of a 48-year-old male, Zhuchkova found that the plexus density in the right atrium was 0.24 percent, and that catecholamines

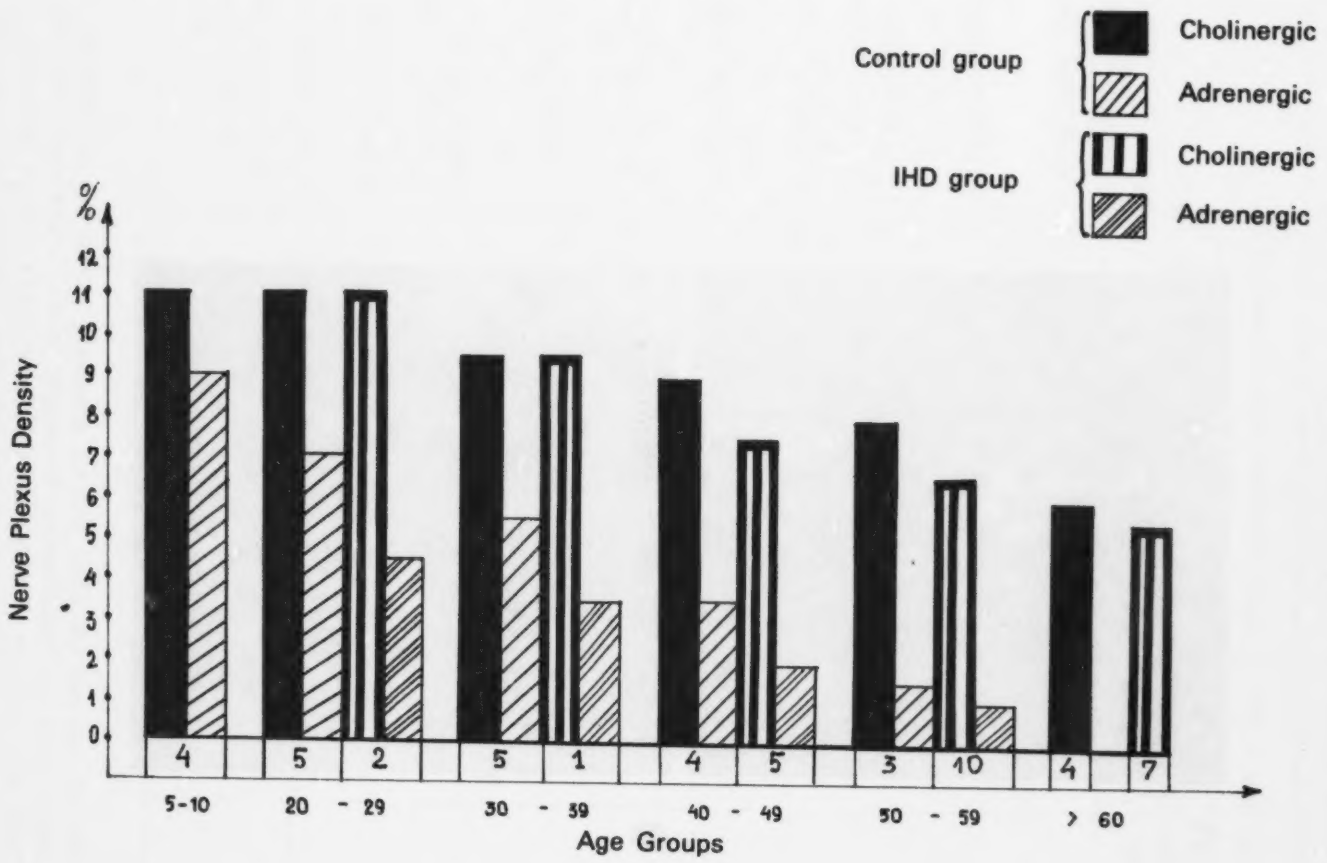

FIGURE 4. Density of cholinergic and adrenergic nerve plexuses in the myocardium of the right atrium in persons of various ages who died in accidents (control group) and from ischemic heart disease (IHD).

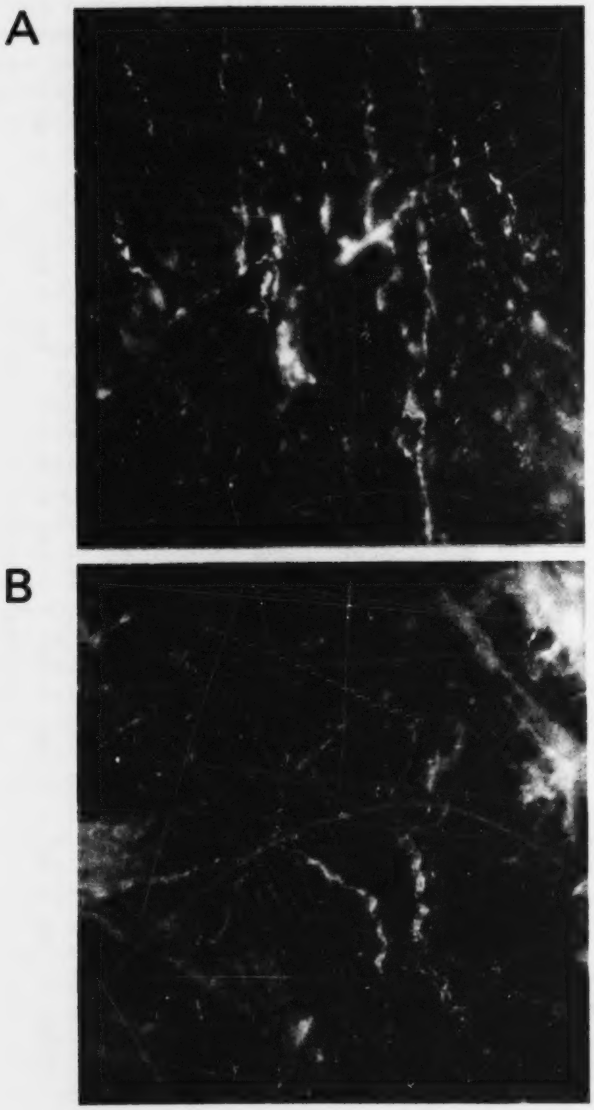

FIGURE 5. Adrenergic nerve plexus, right atrium of the myocardium. A = Male, 31 years of age (control). B = Male, 30 years of age (SCD). Incubation in 2 percent glyoxalic acid. Field 40. Stain Gomal 3.

TABLE 1. Catecholamine and DOPA Content (μg/g tissue) in Various Sections of the Human Myocardium in Cases of Sudden Cardiac Death

Myocardial zones	Ischemic heart disease (IHD)			IHD + myocardial infarction		
	Epinephrine	Norepinephrine	DOPA	Epinephrine	Norepinephrine	DOPA
Left ventricle	163.7 ±35.1	180.5 ±34.5	248.9 ±45.4	146.9 ±32.4	240.5 ±69.5	152.7 ±45.2
Right ventricle	149.0 ±23.1	181.2 ±40.1	274.5 ±46.4	281.9 ±71.4	325.6 ±104.9	226.9 ±51.2
Ventricular septum	112.7 ±25.8	141.5 ±31.9	184.6 ±36.2	186.8 ±63.3	136.7 ±62.1	146.9 ±52.9
Left atrium	207.3 ±39.4	162.5 ±33.8	183.8 ±42.8	135.7 ±45.4	129.8 ±50.7	243.1 ±54.9
Right atrium	155.9 ±27.5	137.9 ±172.7	329.0 ±64.3	221.3 ±80.6	179.4 ±54.9	194.6 ±37.4
Right auricula	141.6 ±39.6	99.8 ±39.2	119.8 ±28.8	104.2 ±41.4	179.6 ±69.3	157.4 ±52.6
Left auricula	55.1 ±14.6	70.9 ±28.2	76.9 ±20.7	46.7 ±20.4	97.9 ±29.6	76.8 ±29.6
Atrioventricular node	90.5 ±28.8	92.8 ±42.2	181.5 ±61.1	99.3 ±36.4	79.3 ±51.8	100.8 ±45.6
Sinoatrial node	81.4 ±20.4	106.2 ±42.2	251.1 ±65.8	133.1 ±61.3	39.8 ±14.8	181.3 ±69.6
Mean values for myocardium as a whole	128.9	130.4	205.6	150.6	156.5	164.5

were lacking in the nerve plexuses in the left atrium and in the intact area of the left ventricle. Only traces of catecholamines were found in the adrenergic plexuses in the right ventricle. Stropus studied the heart of a 27-year-old male who died suddenly and who was autopsied 1.5 hours after death. The findings included an infarction in the left ventricle and complete depletion of the mediators in the adrenergic plexuses of intact areas of the ventricle (figure 6). Thus, the efferent sympathetic plexuses that directly supply

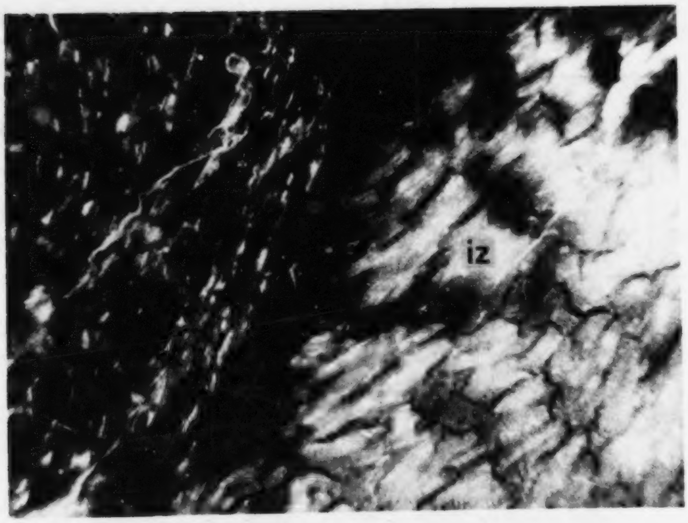

FIGURE 6. Depletion of catecholamines in adrenergic nerve plexuses in the "intact zone" of the left ventricle of a 27-year-old male with a combination of sudden death and myocardial infarction. iz = Infarct zone. Falck-Hillarp-Govyrin method. Field 20. Stain Gomal 3.

cardiomyocytes show depletion or loss of mediators, whereas the cholinergic plexuses are distinguished by a high level of acetylcholinesterase activity. The substantial contrast between the depleted and devastated adrenergic nerve plexuses and the cholinergic terminal plexuses and heart neurons, which are outstanding for the high level of activity of the mediators, is characteristic of the hearts of individuals dying from SCD.

The histochemical characteristics of myocardial cholinergic and adrenergic plexuses apply as well to the perivascular plexuses. We note a relatively lesser depletion of catecholamines in the adrenergic plexuses of the coronary vessels than in the plexuses of the myocardium. The desolation of adrenergic nerve plexuses in the latter is combined with significant disturbances in the microcirculation. Figure 7 shows the phenomena of stasis in myocardial capillaries, as well as areas of catecholamine depletion, arranged mosaically. Areas of capillary hyperemia alternate with areas in which the capillaries are wasted, something seen quite well from the manner in which the reaction to butyryl cholinesterase is organized. Evidently the regional disturbances in the microcirculation of the heart membranes can be noncoronary in origin, connected instead with neuroregulatory metabolic disturbances.

Tsyplenkova studied the myocardium of 16 males aged 24 to 55 years in order to ascertain the ultrastructure of the microcirculatory bed in the heart nerve plexuses in cases of SCD. The materials were taken from the atria, ventricles, and septa no more than 3 hours after death and were processed using generally accepted procedures. Pieces of myocardium from rat cadavers that were kept at ambient temperature for 15, 30, 60, and 180 minutes after killing were studied to delimit autolytic damage to the myocardium from changes that

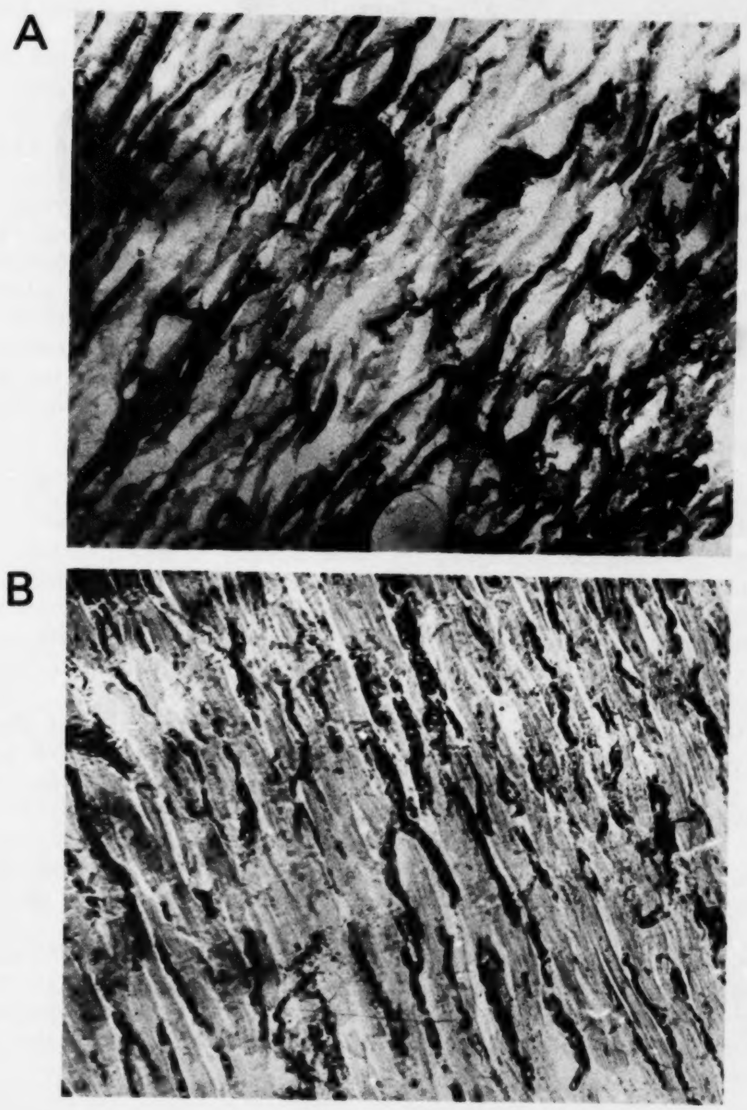

FIGURE 7. Condition of the microcirculatory bed in sudden cardiac death. The photograph shows nonuniform expansion in the capillaries of the left (A) and right (B) ventricular wall in a 48-year-old male. Karnovsky-Roots method. Field 16. Stain 10.

were occurring while the animals were still alive. The research revealed stability of the ultrastructure of the myocardiocytes and the capillaries of the vessels to postmortem autolysis, and the feasibility of studying them over a period lasting up to 3 hours after death.

Study of the myocardium in cases of SCD revealed a definite mosaic pattern of ultrastructural changes in the capillary bed and nerve plexuses in the atria and ventricles. Areas of blood stasis were found with substantial thinning of the capillary wall. The capillary lumen sometimes was covered with protein coagulants, erythrocytes, and more rarely with white blood cells. Found in some of the capillaries was lucidity and edema of endotheliocytes with drastic narrowing or closure of the lumen. An increase in the marginal folds and the appearance of cytoplasmic protuberances were observed in the majority of the myocardial capillaries in cases of SCD. Hypertrophy and hyperplasia of endotheliocytes was observed, the number of micropinocytosic vesicles had increased, and numbers of marginated vesicles, microtubules, and fibrillae appeared. An increase in the quantity of ribosomes and polyribosomes was also noted. The presence of a great many primary lysosomes, usually grouping on four to five organelles, was characteristic (figure 8a).

Nerve conductors and terminals often can be found along the path of capillaries, and close to the cardiomyocytes. Recognizing the lesser stability of nerve tissue to postmortem changes, we made a very thorough electron microscope analysis of the cardiac nerve elements, and always compared these data with data from histochemical analysis. Ultrastructural analysis confirmed the presence of a breakdown of the mediators in the adrenergic nerve conductors, showing a reduction in the number of vesicles at the adrenergic terminals (figure 8b).

Along with direct evidence of the neurodystrophic changes in heart tissue in cases of SCD, obtained from study of nerve structures, there also are indirect signs. It has been established that foci of metachromasia, particularly along the neurovascular bundles and subendocardially, will often be seen in the myocardium of victims of SCD. Preparations stained with toluidine blue were analyzed using an ITsTM-1 measuring color television microscope (16) to establish the degree of metachromatic changes in the various sections of the heart.

The investigation showed that the heart receptor nerve endings undergo what are for the most part reactive changes in SCD. Apparently the disturbances in myocardial metabolism lead to intensive afferent impulsation originating in different heart membranes. This can contribute to the development of periodic heart pain.

Study of the heart in SCD thus leads to the conclusion that a combination of neurodystrophic and hypoxic disturbances in the myocardium is highly important to the genesis of SCD. We undertook certain experiments to study the prerequisites for the development of conditions preceding SCD.

Experiments Revealing Some Aspects of the Pathogenesis of SCD

As known, blood catecholamines increase substantially in stress situations. Yet we see that a characteristic feature in SCD is a deep focal breakdown of the

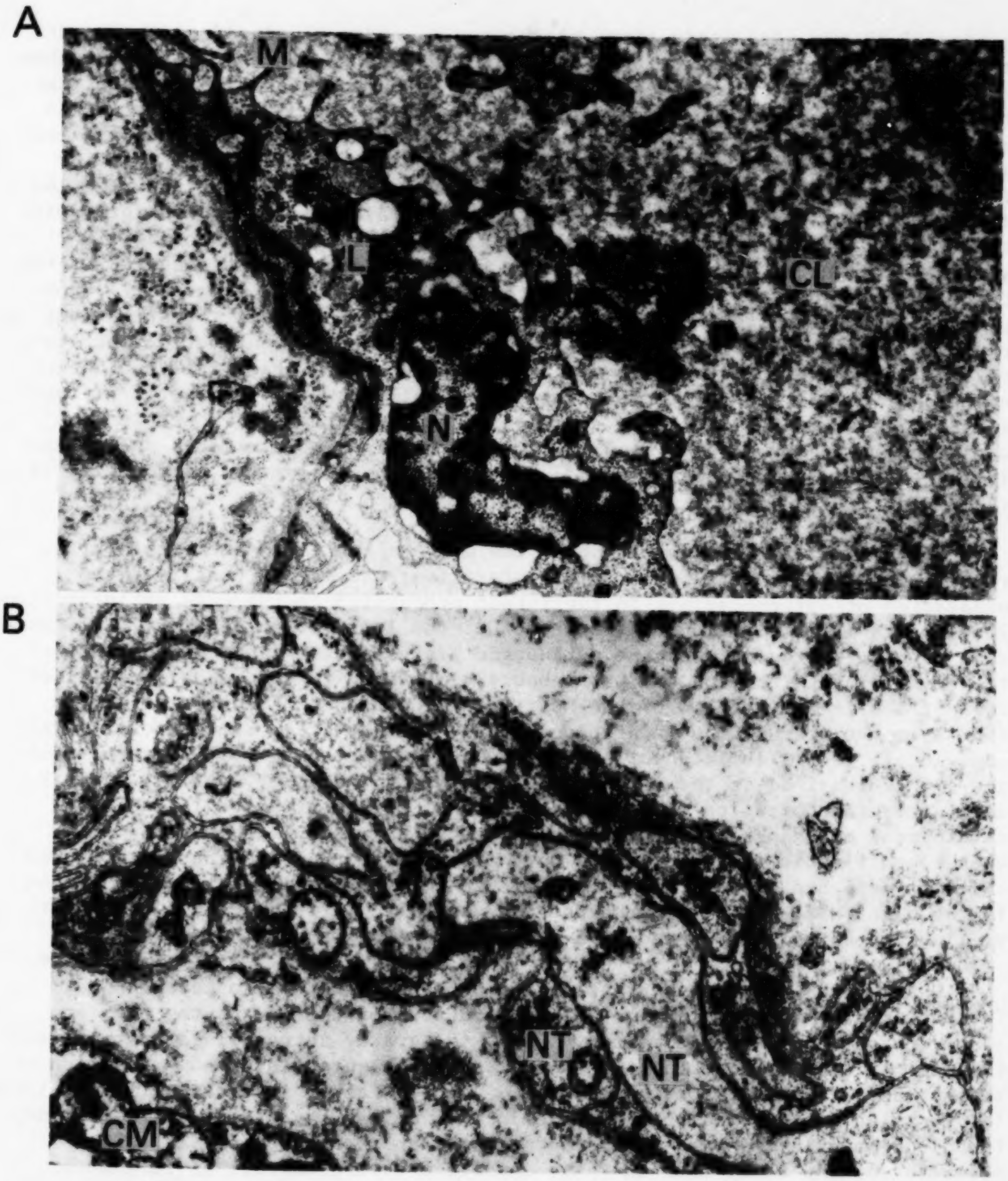

FIGURE 8. Ultrastructure of a human myocardium in sudden cardiac death (SCD). A = Capillary from the myocardium of the right atrium: N = Endotheliocyte nucleus. L = Lysosomes. M = Microvillus. CL = Capillary lumen. B = Nerve terminals located near a cardiomyocyte and devoid of synaptic vesicles: CM = Cardiomyocytes. NT = Nerve terminal. Photograph taken of a heart from a 30-year-old male, 3 hours after SCD. A = Magnification x 16,000. B = Magnification x 20,000.

mediators in the adrenergic nerve plexuses. In order to elucidate the functional significance of this fact, Abraytis and Stropus made parallel histochemical and physiological studies of the striae of human myocardium in the first hours after SCD, and then compared their data with control material from the hearts of victims who had died in automobile accidents. The force of the isometric contraction was determined by the mechanotron method at +25° C after processing the preparations for at least 1 hour. This investigation revealed that the chronoinotropic properties of the myocardial striae in man agree with those found in laboratory animals (17). With addition of epinephrine (10^{-5} g/ml) to the perfused Ringer's solution, a marked increase was observed in the force of the isometric contraction of the myocardial striae (figure 9). Comparison of the results of the histochemical and physiological investigations revealed that the increase in the force of the isometric contraction increased further under the effects of epinephrine in cases where practically no catecholamines, or only traces, were found histochemically in the sympathetic plexuses (figure 9). These observations indicate that a reduction in the content of mediators in the myocardial sympathetic plexuses is accompanied by an increase in the sensitivity of the myocardiocytes to the catecholamines circulating in the blood.

Sosunov studied the condition of the nervous system in the hearts of 60 rabbits with experimental myocardial infarction; this study confirmed the exhaustion of catecholamines in the intact myocardium in the case of infarction. Three hours after ligation of the branch of the left coronary artery, the intensity of the fluorescence of the adrenergic plexuses diminished in the area of the developing infarction, and at 6 hours diminished in the so-called "intact" area of the left ventricle of the heart. At the end of 24 hours the muscle fibers in the area of the infarction displayed light-green luminescence, with individual, weakly luminescent nerve branches among them. Only a few adrenergic fibers remained in the periinfarction area of the left ventricle 3 and 7 days after the operation (figure 10a). These experiments reveal that the development of changes in the adrenergic nerve plexuses is relatively slow when blood flow is impaired in the coronary artery. One can therefore assume that the deep breakdown of the mediators in the adrenergic plexuses described in the clinical data in the presence of myocardial infarction in sudden death victims undoubtedly is caused by a preceding neurodystrophic process.

Changes in the parasympathetic cardiac ganglia were also established. These primarily involved change in the structure of the neurocytes with an electron-dense cytoplasmic matrix. Here, the grainy intracellular network and the laminar complex structure of the mitochondria were disturbed. Reaction of the synapses to the neurocytes was noted 24 hours after infarction (figure 10b). The reaction of the small granular cells (SIF cells) was of interest. Sosunov found cholinergic synapses at the normal body and processes of SIF cells. Revealed for the first time in their cytoplasm were glomeruli of interwoven filaments (with a diameter of 2 μm) near the nucleus. Cells which luminesce orange, together with typical SIF cells which luminesce green, were found during histochemical investigations. The SIF cells described also react to myocardial ischemia. Changes in the intracellular organoids, as well as in the synapses, were also noted.

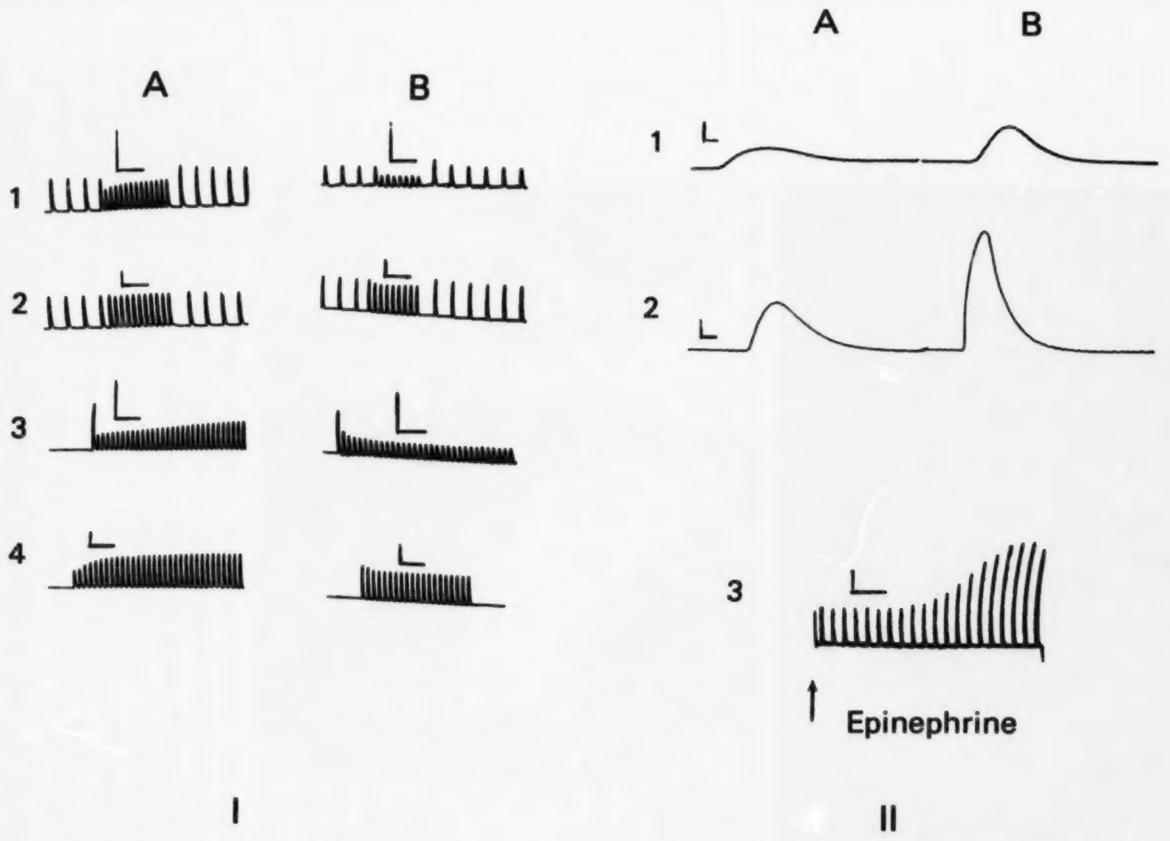

FIGURE 9. I = Chronoinotrophy of isolated stria of the myocardium of papillary muscles (A) and atria (B) from a 30-year-old male 2.5 hours after an accident. 1, 2 = Change in force of contraction upon change from a stimulation frequency of 0.3 pps to a frequency of 1 pps. 1 = Ringer's solution. 2 = Epinephrine 10^{-5} g/ml. Calibration 200 mg, 0.1 sec. 3, 4 = Change in force of contraction after a 3-minute pause (at resting potential). 3 = Ringer's solution. 4 = Epinephrine 10^{-5} g/ml. Calibration same. II = Force of isometric contraction of the atria in Ringer's solution (A) and under the effect of epinephrine 10^{-5} g/ml (B). 1 = 30-year-old male (control). 2 = 74-year-old male (control). 3 = 70-year-old male who died from sudden cardiac death. Calibration: 1 and 2 = 200 mg, 0.1 sec. 3 = 200 mg, 10 sec.

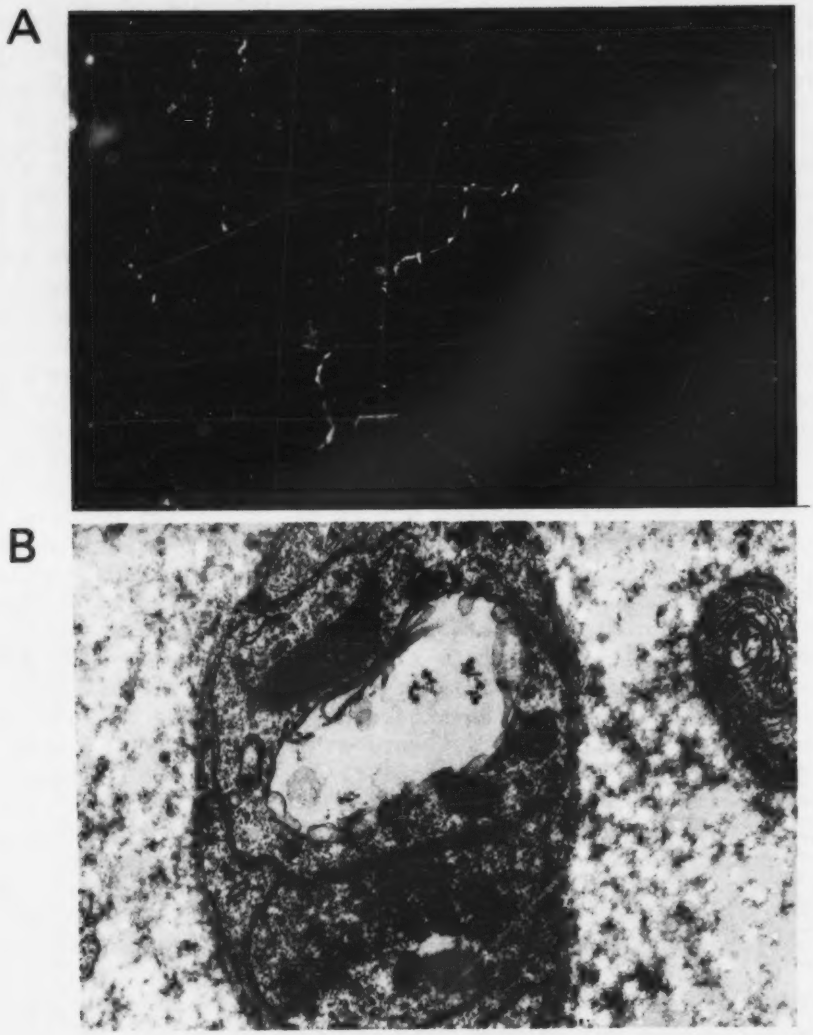

FIGURE 10. A = Condition of the adrenergic nerve terminals in the intact zone of rabbit myocardium after ligation of the left coronary artery branch (72 hours). Incubation in 2 percent glyoxalic acid. Field 40. Stain Gomal 3. B = Ultrastructure of the nerve elements in rabbit cardiac ganglion 24 hours after operation. Axodendritic synapse. Degenerative changes in postsynaptic part. Magnification x 33,000.

Changes in the Extracardiac Nerve Structures in SCD

As pointed out at the USA-USSR First Joint Symposium on Sudden Death in 1977, a reduction in the activity of the adrenergic plexuses, and the reactive-degenerative disturbances of the afferent nerve ends (9), were noted in the sinocarotid reflexogenic zone in cases of SCD. Morphometry of the De Castro type II receptors showed a 220 percent increase in the mean cross-sectional area. Judging from the pathomorphological picture, degenerative changes in the afferent structures developed at least 24-48 hours prior to SCD. Involvement of yet another very important zone, the aortic, has now been established in the pathological process.

A study of the basic sources of adrenergic innervation of the heart in cases of SCD--the cervicothoracic and upper cervical sympathetic ganglia--showed significant changes in the neurons, some of which had degenerated and had been replaced by residual nodules. Two types of changes in the neurons of the sympathetic ganglia were distinguished. Most of the cells contained pigment inclusions. Their bodies were reduced (in midsection area from 520 μm^2 to 420 μm^2) when subjected to Nissl staining. The cytoplasm usually was intensively stained. A second and smaller group of sympathetic neurons was found with edema. Individual neurons of the Dogiel II type were identified upon impregnation of the sympathetic nodes. Study of the sympathetic ganglia after incubation in a glyoxalic acid solution showed reduced adrenergic plexuses in these ganglia. Fluorescing pigmentation was discovered in the cytoplasm of neurons. The ultrastructural changes in the composition of the superior cervical and cervicothoracic sympathetic ganglia in cases of SCD are reminiscent of the picture described by Shvalev and Shustova (18).

Changes in the vagus nerve system were considerably less. Reactive changes in the neurons, with chromatolysis of some neurons, were found in the composition of the nuclei of this nerve in the medulla oblongata. Edema was seen, but degenerative changes were rare. The conducting paths of the extracardiac parts of the vagus nerve were less damaged than the sympathetic nerve in cases of SCD. Here the data concerning the high level of stability of the reflex arches supplying the heart in the vagus nerve system, as compared with the reflex links completed by the spinal ganglia-sympathetic ganglia system, are of interest (19).

The highest autonomic centers which perceive information from the extracardiac reflexogenic zones and from the pressoreceptors and volumoreceptors of the atria are concentrated in the hypothalamohypophyseal neurosecretory system. An increase in the functional activity of this system, according to morphometric, histochemical, and histoenzymatic indices, was seen in cases in which patients died from myocardial infarction in the clinic (20). Evidently, this phenomenon is compensatory and adaptive in nature because entry into the blood of vasopressin and oxytocin increased, along with the catecholamines, thus ensuring maintenance of a definite level of general arterial pressure.

Bogdanovich found dyscirculatory and dystonic changes in the vessels of the microcirculatory bed in cases of SCD. Edema of the white substance of the hypothalamus, focal sponginess of the neuropil, and expansion of the perivascular and pericellular spaces were established (figure 11a); arrangement of the

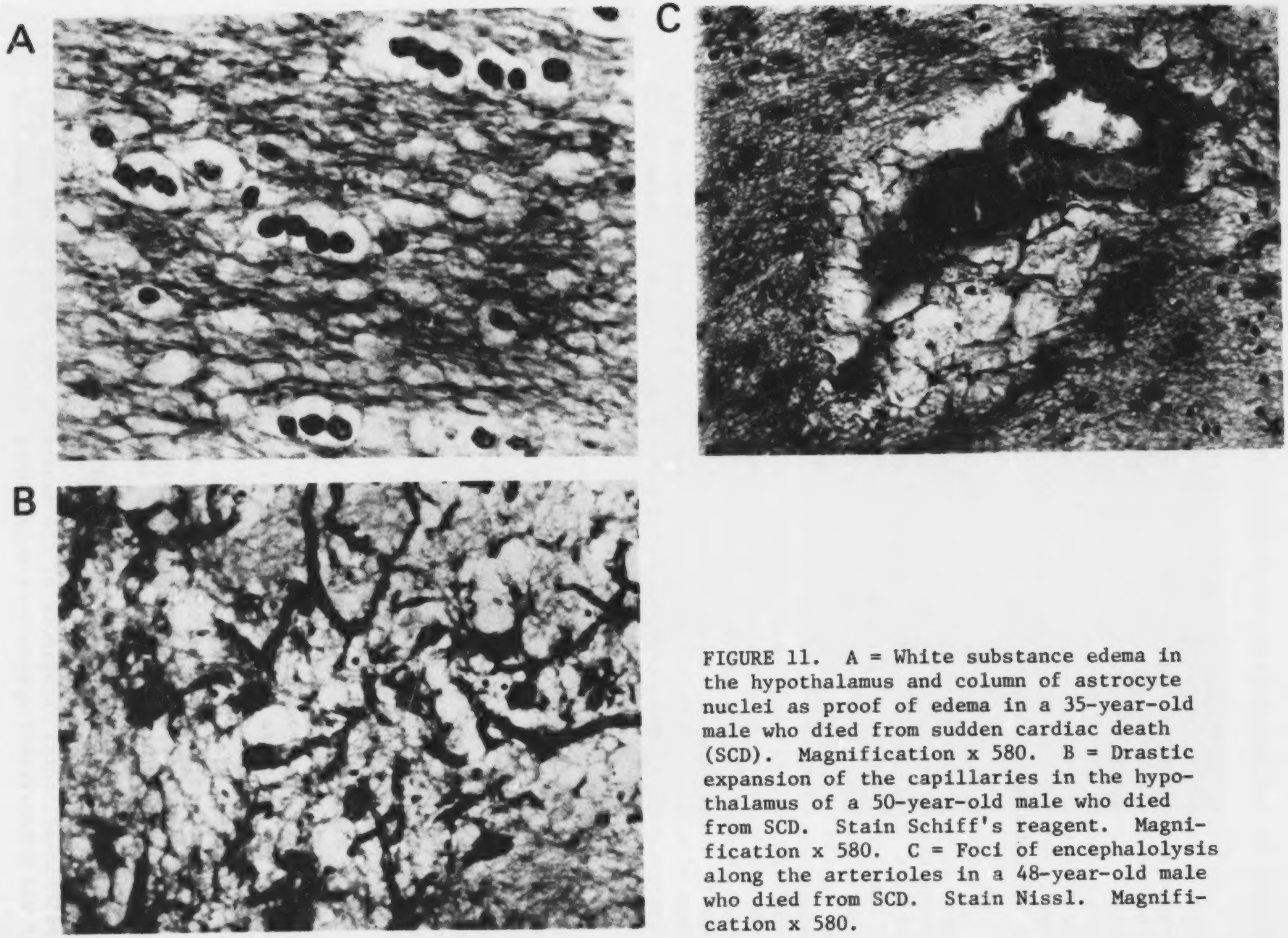

FIGURE 11. A = White substance edema in the hypothalamus and column of astrocyte nuclei as proof of edema in a 35-year-old male who died from sudden cardiac death (SCD). Magnification x 580. B = Drastic expansion of the capillaries in the hypothalamus of a 50-year-old male who died from SCD. Stain Schiff's reagent. Magnification x 580. C = Foci of encephalolysis along the arterioles in a 48-year-old male who died from SCD. Stain Nissl. Magnification x 580.

nuclei of astrocytes in "rouleaux" with broad periglial spaces around them was revealed. The microcirculatory bed of the hypothalamus appeared as if it had been injected (figure 11b). Hemorrhage foci were found in hypothalamus tissue. Sections of encephalolysis were seen around individual arterioles (figure 11c), indicative that the vascular disorders here had been present for some time and were not a manifestation of the terminal period.

The zone in which the large-cell neurosecretory nuclei were located in cases of SCD was usually edematous. Neuron activity in these nuclei was not as pronounced as in myocardial infarction, according to Bogdanovich. Hyperchromatosis of the nuclei of the neurosecretory cells and basophils of their cytoplasm was found, but without changes in size. It can be supposed that such a condition of the neurosecretory centers of the hypothalamus is related to morphological disturbances in the vascular and cardiac reflexogenic zones.

A comparison by Kiseleva and Bogdanovich of the morphological data and the data from investigations of the biochemistry of the hypothalamus is highly interesting. Comparison of the data on catecholamine content in the hypothalamus in cases of SCD and after death in the clinic from myocardial infarction turned up a number of interesting features concerning the concentrations of DOPA, norepinephrine, and epinephrine. It is known (21) that the content of epinephrine in the tissues of the hypothalamus is usually low (6 percent of all catecholamines). There is a sharp increase in the concentration of epinephrine in the hypothalamus in cases of SCD, while the content of DOPA and norepinephrine decrease (figure 12). As known, the increase of epinephrine in the tissues is the cause of accelerated excretion of sodium from the cells into the intercellular space. This aggravates brain edema, and to a significant degree, is responsible for dystonia of the microcirculatory bed. Dystonia of the vessels can be associated with a reduction in the content of norepinephrine in the tissues, which, in turn, has a direct effect on the tone of the vascular wall.

DISCUSSION

Thus, morphobiochemical disturbances of the hypothalamus and changes in the adrenergic and cholinergic components of the nerve system of the heart have been established in cases of SCD. Signs of insufficiency in the processes of adaptative and trophic innervation of the heart were found, which could lead to the development of SCD because of complex disturbances in the regulatory mechanisms of the cardiovascular system. The research of Borchard (22) on the cardiac nervous system is of interest in terms of the hypertrophic processes taking place in the heart.

The work of the Anichkov school should be recalled in this respect. This group of investigators has pointed out that the neurodystrophic changes in the myocardium and in the walls of the blood vessels are under the effects of the hypothalamus and reflexogenic vascular zones (23,24). Disturbance of neurons in the medulla oblongata and in the spinal cord--in the sympathetic, vagal, and spinal ganglia--was found, as well as changes in the intracardiac adrenergic and cholinergic nerve plexuses (25). This led to the contemplation of treatment and preventive measures using individualized prescription of neurotrophic drugs in order to prevent the development of visceral neurodystrophic changes.

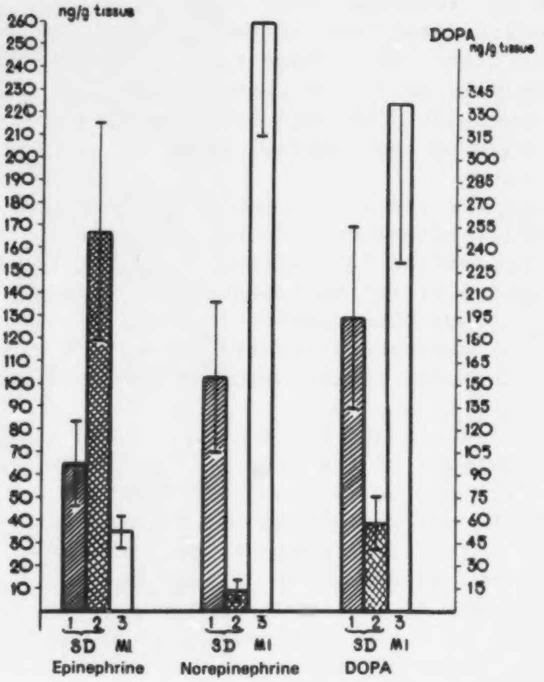

FIGURE 12. Catecholamine content in the hypothalamus tissue in cases of sudden death and myocardial infarction. SD = Sudden cardiac death. MI = Myocardial infarction. 1 = SD without MI. 2 = SD with MI.

It should be recalled that Lown's conception (26) of the essence of ciliary arrhythmia occurring after experimental stimulation of the hypothalamus and simultaneous ligation of the coronary artery, drew critical comments at the USA-USSR First Joint Symposium on Sudden Death in terms of underestimating the damage to the myocardial afferent and efferent nerve plexuses. Still, his new data concerning the importance of the vagus nerve in ensuring the electrical stability of the ventricles are interesting. These data require particular attention in view of the inadequate study thus far of the vagal branches in these parts of the heart. Future modeling of conditions approaching those of SCD will necessitate even more thorough study of neuromorphological changes at all levels of the nervous system, including the entire systems of the sympathetic and parasympathetic parts of the autonomic innervation of the heart.

SUMMARY

Complex morphobiochemical research has established the combination of lesions of the cardiac nerve plexuses and microcirculatory bed in cases of SCD. Quantitative indices for the mottling of the lesions of the myocardial adrenergic plexuses, and the accompanying biochemical damage to the content of cardiac catecholamines, were established. A relatively high level of activity of

mediator content was found in the cholinergic plexuses against a background of drastic deficiencies of mediators in the myocardial adrenergic nerve plexuses.

Discovery of the staging of the increase in the development and involution of the cholinergic and adrenergic nerve plexuses of the cardiovascular system (27) made it possible to compare the cardiac nervous system under normal conditions and in cases of SCD. Data from cases of SCD were compared with the dynamics of change in the adrenergic and cholinergic nerve plexuses of the human heart from the standpoint of an increase under normal conditions, which was established for the first time. At the same time, new findings that involution of the mediators in the adrenergic plexuses progresses under normal conditions after age 40 in the human heart, while cholinergic plexuses are relatively stable, were taken into consideration. In cases of SCD, the content of mediators in the cardiac adrenergic plexuses is lower than is normal by a factor of 3 to 4, and death precedes a deep reduction in myocardial adaptational and trophic innervation.

Study of the reaction of myocardial striae from the atria and ventricles of the human heart after SCD revealed an increase in their sensitivity to the effect of epinephrine. Thus, it was found that, after considerable depletion of mediators in the intracardiac plexuses, that is, after increasing myocardial sympathectomy, the cardiomyocytes of the human heart develop increased sensitivity to epinephrine entering the heart with the blood. It is important to note that Beskrovnova et al. saw an increase in the excitability of the myocardium after sympathectomy (see her report in these proceedings), and there was a predisposition to the development of fibrillation so characteristic of SCD.

Changes in neurons and gliacytes found in the sympathetic ganglia preceded SCD, judging from the extent of their changes. Some of these changes may have developed as early as the beginning of the mediator stage, while others appear to have developed in the postnatal period. It is known that the sympathetic ganglia are some of the most vulnerable parts of the autonomic nervous system and that changes in their neurons can often be seen under "normal" conditions (28). Proliferation in the ganglia of the glial and connective tissue in sudden death is indicative of chronic processes, at the same time that degeneration and disintegration of nerve plexuses, particularly afferent ones, point to the presence of acute pathological processes directly preceding SCD.

Our work revealed a mutual causal effect between damage to the nervous system and the microcirculatory bed of the myocardium, that is, pointing to the associative nature of neurovascular lesions in SCD. Capillaries were exposed in the reactions to pseudo and true cholinesterase, and in electron microscopy. Mottling in changes in the microcirculatory bed was detected, and disturbances of capillaries in various parts of the myocardium that had occurred while alive were found. Damage to the blood-carrying channels in SCD can evidently be primary, secondary, and even other neurodystrophic processes (29-31). Our observations showed that, in cases when SCD is accompanied by myocardial infarction, development of SCD can be preceded by changes in the nerve plexuses, particularly in the adrenergic plexus. Experimental observations, in turn, indicate definite dynamics in the development of postinfarction lesions of the myocardial nerve plexuses, and of the ultrastructure of the intracardiac neurons and SIF cells.

The vagus nerve system and its central formations in the medulla oblongata, as well as the intracardiac nerve ganglia and plexuses, show relatively fewer changes compared with the sympathetic part of the autonomic nervous system. Reactive and destructive changes in the afferent structures were found in the vascular reflexogenic zones (sinocarotid and aortic).

Marked dystonic changes in the microcirculatory bed, with edema in the area of the nerve conductors, were found in the hypothalamus. Foci of hemorrhages and sections of encephalolysis were noted. The zone in which the neurosecretory nuclei are located was edematous. Activity of their neurons was low. Hypothalamus disturbances, in combination with the above-described changes in cardiac nerve plexuses, undoubtedly cause insufficiency in the adaptational and trophic regulation of the myocardium and its vessels. Biochemical study found a relatively high content of epinephrine in the hypothalamus in cases of SCD, while the content of DOPA and norepinephrine were reduced compared with the corresponding analyses of the hypothalamus of patients who died in the clinic after an infarction.

This research, which revealed the insufficiency of the adaptational and trophic function of the cardiac autonomic nervous system characteristic of SCD among those who died, leads to the practical conclusion that it is necessary to conduct preventive research on the state of the sympathetic and parasympathetic parts of the nervous system in persons who are found to have a complex of risk factors. This is necessary for the preventive use of certain neurotropic drugs and mediator precursors.

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PARASYMPATHETIC NEURAL EFFECTS ON VENTRICULAR ELECTRICAL STABILITY

Bernard Lown and Richard L. Verrier

ABSTRACT. Enhanced vagal tone, whether induced by vagus nerve stimulation or by direct muscarinic receptor stimulation, significantly reduces vulnerability to ventricular fibrillation in the normal and ischemic myocardium. This protective action results from an antagonism of the profibrillatory effects of enhanced neural and humoral input to the heart. These mechanisms appear to operate in the conscious animal as well as in the anesthetized animal. These findings have readily applicable clinical implications.

INTRODUCTION

Vagal influence on ventricular myocardial excitability is a subject under current reappraisal. The prevailing view has been that vagal innervation does not extend to the ventricular myocardium. It has been a commonly held clinical view that, if a tachycardia responds to cholinergic measures, the site of impulse formation is supraventricular. Considerable current evidence, however, indicates that parasympathetic neural influences alter ventricular electrical properties (1-5). Several groups of investigators have demonstrated that vagus nerve stimulation significantly influences ventricular excitability (6) and vulnerability (1,3,7). Such effects can be mediated by the rich cholinergic innervation of the specialized conduction system found in both the canine and human heart (2).

We demonstrated that the effect of the vagus on vulnerability to ventricular fibrillation (VF) is contingent on the preexisting level of cardiac sympathetic tone (3,8). This thesis derives from a number of experimental findings. For example, vagus effect is enhanced in the thoracotomized animal

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which exhibits augmented sympathetic tone to the heart and also during sympathetic nerve stimulation and catecholamine infusion. Such vagus action on cardiac vulnerability is obviated during beta-adrenergic blockade.

There is as yet uncertainty as to whether vagus nerve activity alters ventricular vulnerability during acute myocardial ischemia. Kent, Epstein, and coworkers (1,2) found that vagus nerve stimulation significantly increased the VF threshold and decreased susceptibility to fibrillation in the ischemic canine heart. Subsequently, Corr, Gillis, and coworkers (9,10) observed that the presence of intact vagi protected against VF in chloralose-anesthetized cats during left anterior descending coronary artery ligation, but was not beneficial during right coronary artery obstruction. Yoon et al. (7) and James et al. (11) were unable to demonstrate any effect of vagus nerve stimulation on VF threshold during left anterior descending coronary artery occlusion in dogs. Corr and coworkers (12) have even found that cholinergic stimulation may exacerbate rather than ameliorate the arrhythmias which ensue upon release of occlusion, with attendant reperfusion of the ischemic myocardium.

A pertinent unanswered question is whether tonic vagal activity modulates ventricular electrical stability in the conscious state. While data derived from nerve stimulation and drug infusion experiments in anesthetized animals yield valuable information, such approaches are partially artifactual and need corroboration in the intact, conscious organism. Study of animals in the awake state has been hindered until recently by the absence of suitable biologic models for assessing vulnerability to VF. However, this difficulty has been circumvented by utilizing the repetitive extrasystole threshold which provides a reproducible index of vulnerability and thereby obviates the need for inducing VF and for attendant resuscitative procedures (8,13,14).

The purpose of the following investigations was threefold: (a) to examine the influence of vagus nerve stimulation and direct muscarinic receptor stimulation with methacholine on vulnerability to VF during acute myocardial ischemia and reperfusion, (b) to determine whether tonic vagal activity modulates ventricular vulnerability in the conscious state, and (c) to evaluate whether the observed findings in animals have any clinical relevance.

MATERIALS AND METHODS

Anesthetized Animal Studies

General procedures

Studies were performed in 54 healthy mongrel dogs weighing 9 to 25 kg. A minimum of 5 days before study, a left thoracotomy was performed through the fourth intercostal space under general pentobarbital anesthesia. The heart was exposed and an occlusive balloon catheter system was placed around the left anterior descending coronary artery at the level of the left atrial appendage. The patency of the balloon was tested by inflating it at the time of operation. The catheter was exteriorized through a subcutaneous tunnel in the nape of the neck.

On the day of study, the dogs were anesthetized with alpha-chloralose, 100 mg/kg intravenously. Artificial ventilation was maintained via a cuffed endotracheal tube attached to a Harvard constant-volume pump delivering a mixture of room air and 100 percent oxygen. This mixture was adjusted to maintain arterial pO_2 between 125 and 225 mm Hg. Arterial pH was maintained in a range of 7.30 to 7.55. Abdominal aortic pressure was measured with a large lumen catheter inserted through a femoral arteriotomy and connected to a Statham P23Db pressure transducer. The electrocardiogram (ECG) was recorded from a unipolar right ventricular endocavitary lead.

Cardiac testing

Bipolar transvenous fixed rate right ventricular pacing was used throughout the experiments. A bipolar catheter (Medtronic no. 5819), inserted through the right jugular vein and positioned at the apex of the right ventricle under fluoroscopic control, served for both pacing and pulsing. Pacing was accomplished by a stimulus 50 to 100 percent above threshold at an interval of 333 to 300 msec, corresponding to respective ventricular rates of 180 to 200/minute.

Ventricular fibrillation thresholds were determined using a single stimulus having a duration of 10 msec and this determination was accomplished as follows: Electrical diastole was initially scanned with a pulse of 4 milliampere (mA) in 10 msec intervals beginning at the boundary of the effective refractory period and terminating beyond the T wave. The current was then increased in 2 mA steps and scanning was continued for 3 seconds after each stimulus discharge. The lowest stimulus intensity that elicited VF was taken as the VF threshold.

The following experimental protocol was used: The left anterior descending coronary artery was completely occluded for 10 minutes by inflating the previously implanted balloon catheter. During occlusion, the VF threshold was assessed at approximately 1 minute intervals until recovery and preocclusion values were reestablished. The balloon catheter was abruptly deflated 10 minutes after occlusion. Two occlusions with and without an experimental intervention were performed, separated by an interval of at least 20 minutes (15,16).

Defibrillation was accomplished, usually within 3 seconds by a direct current, 50 to 100 watt-second capacitor discharge from a Lown cardioverter. This resuscitative procedure in closed-chest dogs does not significantly affect the stability of the VF threshold.

Vagus nerve stimulation

The cervical vagosympathetic trunks were sectioned bilaterally 2 cm below the level of the carotid bifurcation. The distal cut ends of the vagi were mounted on insulated bipolar electrodes. Nerve stimulation was accomplished using rectangular pulses of 5 msec duration, 20 Hz and 3-15 volts. Stimulation voltages were independently adjusted so that asystole was produced with either right- or left-sided vagal excitation. Ventricular fibrillation threshold was determined before, during, and after bilateral vagal stimulation. Heart rate was maintained constant at 200 beats/minute during threshold testing.

Methacholine infusion

The muscarinic agent, acetyl-(D,L)-beta-methylcholine chloride (J.T. Baker Company) in normal saline solution was infused intravenously at a rate of 5 μg/kg/min using a Harvard infusion pump. Maximal effects on VF threshold were noted after 30 minutes of infusion at which time coronary artery occlusion and release sequence was carried out. The drug was infused throughout this procedure.

Conscious Animal Studies

These investigations were performed in 18 healthy mongrel dogs weighing between 10 and 15 kg.

Cryogenic vagus nerve blockade

A technique was developed to produce reversible cold blockade of cardiac vagal activity. To accomplish this, a 3 to 4 cm length of exteriorized vagosympathetic trunk was enclosed in a tube of cervical skin. Thus, "vagal loops" were created on each side of the neck which separated the segments of enclosed nerve isolated from other cervical structures. Cooling probes could then be placed around the vagal loops to induce reversible blockade of neural traffic. The relative contributions of vagal efferent and afferent activity to the effects observed were determined by comparing the results of vagal cooling to those obtained in response to selective vagal efferent blockade with intravenous atropine.

Cardiac testing

The repetitive extrasystole (RE) threshold method was used to determine vulnerability to VF as previously described (13,17). Briefly, the vulnerable period threshold was assessed as follows: With heart rate maintained at 220 beats/minute, scanning for the RE threshold was commenced with a stimulus intensity of twice the mid-diastolic threshold starting 30 msec beyond the refractory period. The test stimulus was delivered progressively earlier by 5 msec intervals until the refractory period was encountered. If no RE resulted, the stimulus intensity was increased by 2 mA and the scanning procedure was repeated. The RE threshold was taken to be the minimum current intensity at which RE occurred in two out of three trials.

Psychologic environment

To study the influence of sympathetic-parasympathetic interaction in the conscious state, dogs were exposed to a stressful environment which augments adrenergic input to the heart (14,18).

The stressful environment consisted of a Pavlovian sling in which the animal was restrained and movement was limited. Cables were connected to the cardiac catheters so that a continuous oscilloscopic display of the ECG was obtained and pacing and test stimuli could be delivered. A single 5 msec shock from a cardioverter was delivered via copper plates (80 cm^2) fastened across the thorax. The dog remained in the sling for 10 minutes before the synchronized transthoracic shock was administered and for 10 minutes following the shock. The shock was delivered on 3 consecutive days. On the fourth day, the dogs received no further electric shock. The effect of this environment on the vulnerable period threshold before and after blockade of vagal efferent activity with atropine (0.05 mg/kg) was examined at this time.

Statistical analyses were made using Student's paired *t*-test. All values are expressed as mean ± SEM.

RESULTS

Influence of Cholinergic Stimulation on Vulnerability to VF During Myocardial Ischemia and Reperfusion

The effect of vagus nerve stimulation on VF threshold prior to and during a 10-minute period of left anterior descending coronary artery occlusion followed by abrupt release was studied in 24 chloralose-anesthetized dogs. In the absence of vagus nerve stimulation, coronary artery obstruction and release-reperfusion resulted in a significant reduction in fibrillation threshold (figure 1). The decrease in threshold occurred within 2 minutes following occlusion and persisted for 5 to 7 minutes. Thereafter, the threshold rapidly returned toward the preocclusion value. Upon release of occlusion, the fall in threshold was nearly immediate, occurring within 20 to 30 seconds, and transient, persisting less than 1 minute. Vagus nerve stimulation significantly increased the VF threshold prior to coronary artery occlusion (from 17 ± 2 mA to 33 ± 4 mA, $p < 0.05$) and blunted the decrease in threshold associated with myocardial ischemia (6 ± 1 mA compared to 18 ± 4 mA, $p < 0.05$). No protective influence of vagal stimulation was observed during reperfusion (5 ± 1 mA compared to 3 ± 1 mA).

The effect of selective muscarinic receptor stimulation with methacholine on vulnerability was investigated in 10 animals. Methacholine infusion produced results which were qualitatively similar to those observed in response to vagal stimulation. Namely, the drug increased fibrillation threshold prior to and during coronary artery occlusion but was ineffective in altering the fall in threshold associated with reperfusion (figure 2).

Effect of Vagus Nerve Excitation on Susceptibility to Spontaneous VF During Myocardial Ischemia and Reperfusion

The influence of vagal stimulation on the occurrence of spontaneous VF during occlusion of the left anterior descending and septal coronary arteries was investigated in 16 additional dogs. Heart rate was maintained constant

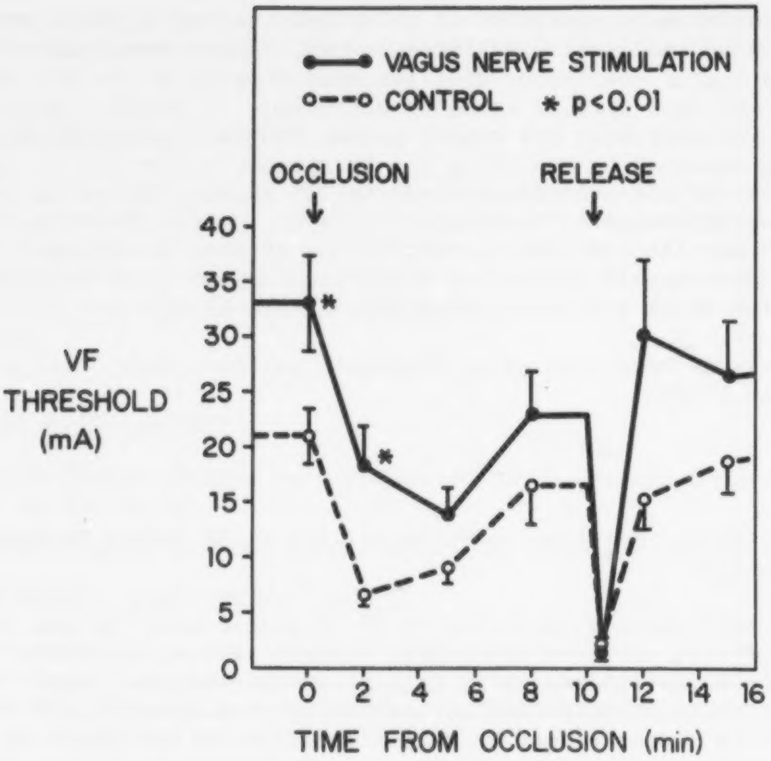

FIGURE 1. Influence of vagus nerve stimulation on ventricular fibrillation (VF) threshold during a 10-minute period of left anterior descending coronary artery occlusion followed by abrupt release. Study of 24 chloralose-anesthetized dogs. Data points present mean ± SEM. Partial protection against vulnerability to VF was conferred by vagal stimulation during coronary occlusion. No protection, however, was afforded during reperfusion.

at 180 beats/minute by ventricular pacing. In the absence of vagus nerve stimulation, coronary artery occlusion provoked VF in 7 out of 10 dogs (70 percent), whereas during concurrent vagal activation VF developed in only 3 out of 10 animals (30 percent, $p < 0.05$) (figure 3). No significant protection was afforded against VF during reperfusion.

Tonic Vagal Influence on Vulnerability in Animals Exposed to Psychologic Stress

This problem was examined in 10 conscious dogs in which the vagi had been chronically exteriorized in a tube of cervical skin. Neural traffic in the vagosympathetic trunk was blocked reversibly by cooling probes placed around the cutaneous vagal loops. Cryogenic blockade of the left and right vagal loops increased heart rate from 95 ± 5 beats/minute to 115 ± 7 and 172 ± 16 beats/minute, respectively. When both vagal loops were cooled simultaneously,

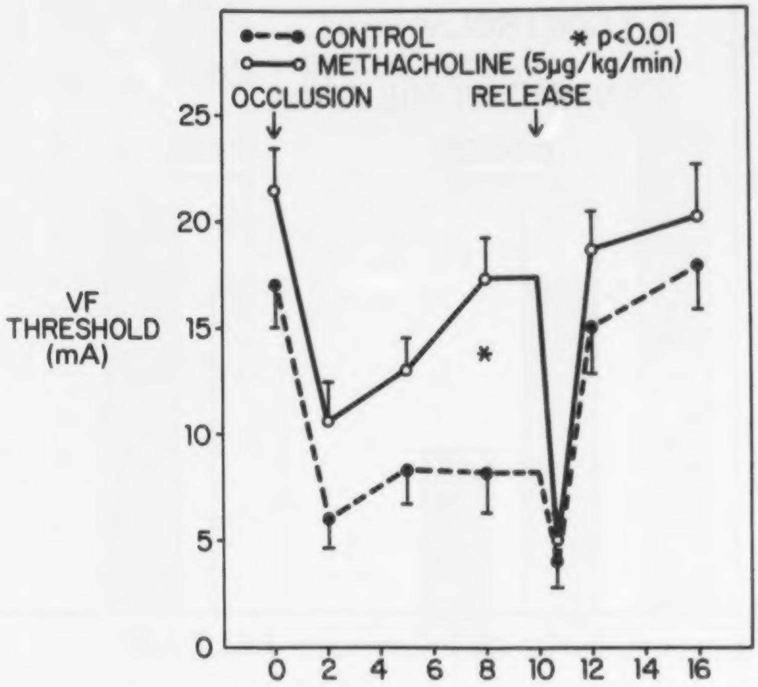

FIGURE 2. Effect of direct muscarinic receptor stimulation with methacholine on VF threshold during coronary artery occlusion and reperfusion in 10 chloralose-anesthetized dogs. As was observed with vagus nerve stimulation (see figure 1), methacholine infusion lessened ischemia-induced vulnerability but was ineffective during reperfusion.

heart rate increased to 208 ± 20 beats/minute. All changes in heart rate were statistically significant at the $p < 0.01$ level (figure 4).

The effect of selective vagal efferent blockade with atropine on the RE threshold was studied in eight conscious dogs exposed to a stressful environment consisting of a Pavlovian sling in which they received a mild cutaneous shock. Prior to interruption of vagal input to the heart, the RE threshold was 15 ± 1 mA. Following atropine administration (0.05 mg/kg), the threshold decreased significantly to 8 ± 1 mA (47 percent reduction, $p < 0.0001$) (figure 5). This effect occurred independently of a heart rate change since rate was maintained constant at 200 beats/minute during cardiac electrical testing. Vagal blockade with atropine did not significantly alter RE threshold in the nonstressful cage environment (22 ± 2 mA compared to 19 ± 3 mA, before and after the drug, respectively).

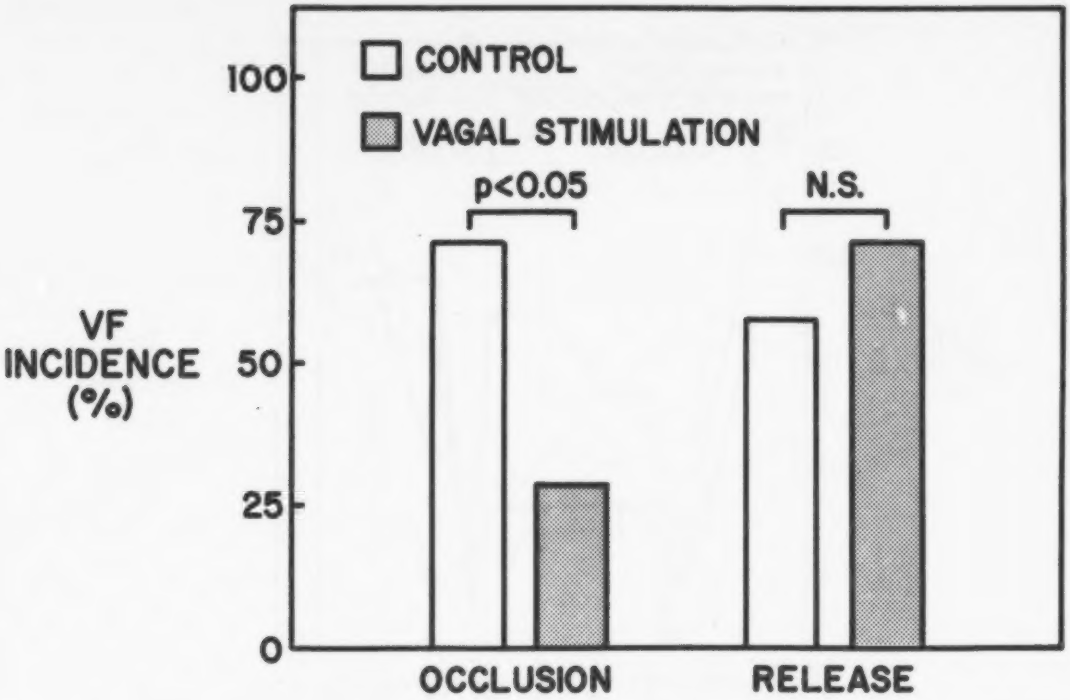

FIGURE 3. Influence of vagus nerve stimulation on susceptibility to spontaneous VF during a 10-minute period of left anterior descending and septal coronary artery occlusion followed by reperfusion. Consistent with the effects of parasympathetic interventions on vulnerability during myocardial ischemia (see figures 1 and 2), vagal stimulation decreases susceptibility to spontaneous VF during coronary occlusion but not release.

DISCUSSION

Considerable evidence has now been amassed indicating that parasympathetic influence directly affects chronotropic, inotropic, and excitable properties of the ventricular myocardium. Whether this influence is of sufficient magnitude to afford some protection against VF in the ischemic heart is much less certain. Furthermore, there is a paucity of information relating to the significance of vagal nerve input on the predisposition to VF under the two diverse conditions which probably play a role in triggering sudden cardiac death in man, namely abrupt coronary artery obstruction and its release with ensuing reperfusion. The magnitude of tonic vagal activity has hitherto been undefined. An additional unresolved question is whether such tonic parasympathetic neural traffic can alter the change in ventricular vulnerability resulting from minor psychophysiologic stresses. The present investigation has shed some light on these questions.

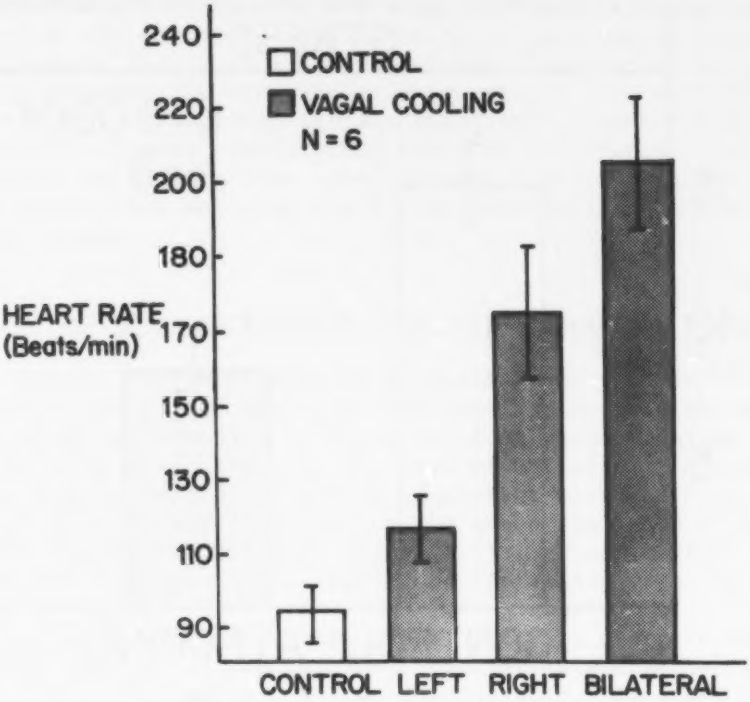

FIGURE 4. Heart rate response to vagus nerve cooling in conscious dogs. Cryogenic blockade of cutaneous loops containing either the left or right cervical vagus resulted in substantial increases in heart rate. The rate effects, however, were most marked during bilateral vagal cooling.

Influence of Vagal Stimulation During Myocardial Ischemia and Reperfusion

We found that intense parasympathetic stimulation by electrical excitation of the decentralized vagi or by direct muscarinic receptor stimulation with methacholine reduces vulnerability to VF during acute myocardial ischemia in the canine heart. This view is supported by the observations that cholinergic stimulation significantly lessened both the fall in VF threshold and the incidence of spontaneous VF during coronary artery occlusion. These effects were independent of heart rate changes, since heart rate was maintained constant by pacing. No such salutary effects on vulnerability from vagus nerve stimulation or muscarinic receptor activation were observed during reperfusion.

What then is the possible basis for these differing effects of the vagus during myocardial ischemia and reperfusion? It may be that disparate mechanisms are responsible for susceptibility to VF during coronary artery occlusion and reperfusion (14,16). Reflex activation of cardiac sympathetic activity appears to play a major role in vulnerability during acute coronary artery occlusion (19,20). Supporting this thesis is the time course of changes in adrenergic input to the heart which corresponds closely with the reduction in vulnerable period threshold and spontaneous occurrence of VF during coronary

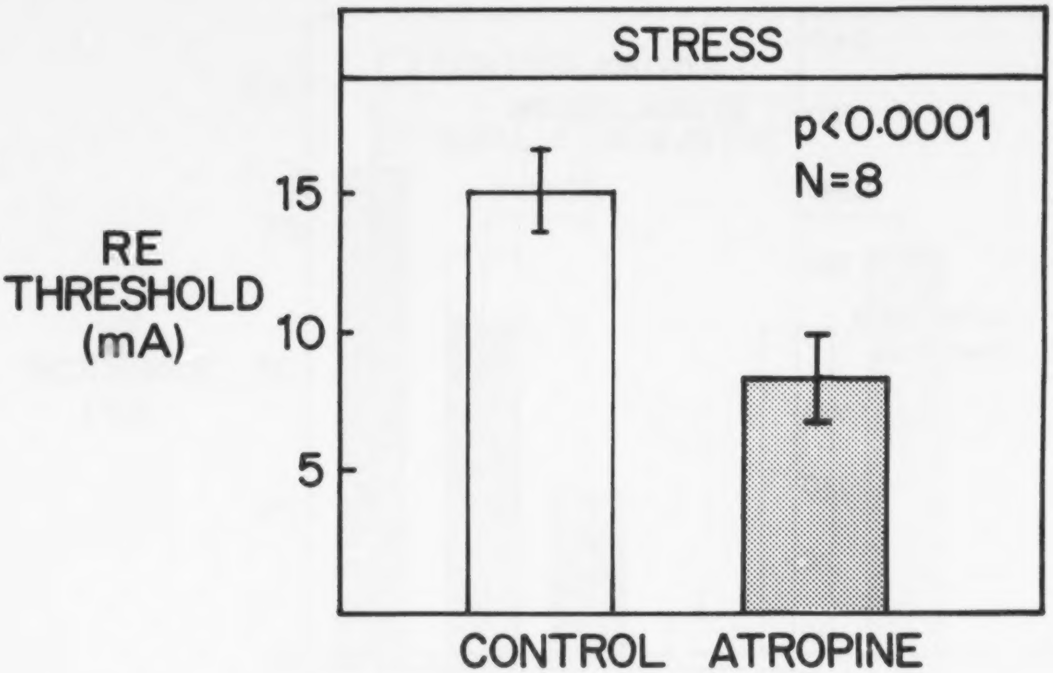

FIGURE 5. Influence of atropine (0.05 mg/kg) on repetitive extrasystole (RE) threshold in conscious dogs exposed to a stressful Pavlovian sling environment. Blockade of vagal efferent activity with atropine substantially reduced the vulnerable period threshold indicating an enhanced propensity for VF.

artery occlusion (15). When sympathetic input to the heart is blunted by surgical (21) and pharmacologic means (16,22) significant protection is afforded against ischemia-induced VF. Parasympathetic stimulation thereby decreases vulnerability to VF during coronary artery occlusion by antagonizing the fibrillatory influence of enhanced adrenergic activity. This salutary effect of cholinergic stimulation may result from inhibition of the release of norepinephrine from sympathetic nerve endings (23) and from attenuation of the response to catecholamine action at receptor sites (24).

Vulnerability during reperfusion, however, appears to be due to extra-adrenergic factors. Available evidence indicates that washout products of cellular ischemia and necrosis may be involved (25). It has been demonstrated that, if blood flow is gradually restored to the ischemic myocardium (26,27), or if perfusion is maintained with deoxygenated fluid, the incidence of ventricular arrhythmias on reinstitution of flow is substantially reduced (26,28,29). The finding that VF ensues within seconds after reinstitution of coronary arterial flow also argues for washout of metabolic products (16,25). Interruption of cardiac sympathetic input by surgical or pharmacologic interventions is ineffective in preventing release VF. Since cholinergic maneuvers exert a protective effect solely through antiadrenergic action, this may in part explain their failure to reduce vulnerability to VF during reperfusion.

The profound influence of the vagus on heart rate may substantially alter the effect of the vagus on susceptibility to ventricular arrhythmias. For example, Kerzner et al. (30) have shown that vagal stimulation does not completely suppress the arrhythmias which result from myocardial infarction. On the contrary, these investigators found that enhanced vagus activity or acetylcholine infusion consistently elicited ventricular tachycardia during the quiescent arrhythmia-free phase of myocardial infarction in dogs. This proarrhythmic action was completely rate-dependent and could be prevented by pacing the heart.

Tonic Vagal Influence on Vulnerability in the Conscious State

The results of the present study indicate that there is a high level of vagal tone to the canine heart in the conscious resting state. Cryogenic blockade of either the left or right cervical vagus resulted in substantial changes in heart rate; however, the predominant effect occurred during right-sided blockade (figure 4). This is consistent with the fact that the right vagus exerts a dominant influence on the sinoatrial node with some overlapping effect attributable to the left vagus (2,31). Thus, maximal increase in heart rate occurred during combined right- and left-sided vagal cooling.

Having established that tonic vagal activity exerts a profound influence on pacemaker tissue, it was pertinent to examine whether an effect of ventricular electrical properties was also demonstrable. In these experiments, atropine was employed to block selectively vagal efferent activity to the heart. Dogs were exposed to an aversive Pavlovian sling environment to augment cardiac sympathetic tone (18). This experimental design permitted study of the influence of sympathetic-parasympathetic interaction on vulnerability in the awake animal. We found that administration of a relatively low dose of atropine (0.05 mg/kg) resulted in a nearly 50 percent reduction in the vulnerable period threshold. The implication is that in the stressed animal a considerable level of vagal tone is present which partly annuls the profibrillatory influence of aversive psychophysiologic stimuli.

Also, within this setting, the protective effect of the vagus was most likely due to an antagonism of adrenergic mechanisms. This supposition is supported by two sets of observations. First, our previous investigations demonstrate that ventricular vulnerability in this stress model correlates closely with circulating catecholamine levels (18) and that interruption of cardiac sympathetic input to the heart by beta-adrenergic blockade or sympathectomy substantially blunts the stress-induced changes in vulnerability (32). Second is the finding of DeSilva et al. (17) that enhancing cardiac vagal tone by administration of morphine to dogs in the stressful sling environment increases the vulnerable period threshold to the value observed in a nonstressful cage setting. When vagal efferent activity was blocked by atropine a major component of protective effect resulting from morphine was abolished. Administration of morphine in the nonstressful cage failed to alter the vulnerable period threshold, presumably because in this environment cardiac adrenergic input was low.

These findings indicate that central activation of the vagi, whether occurring spontaneously or induced pharmacologically, protects against myocardial susceptibility to VF during stress. This beneficial action most likely is due to antagonism of enhanced adrenergic input to the heart.

CLINICAL IMPLICATIONS

More than 40 years ago it was demonstrated that administration of the cholinergic drug, acetyl beta methylcholin chloride, prevented ventricular arrhythmias in man that resulted from the infusion of adrenalin (33). Recently, a number of studies have reported that parasympathetic maneuvers such as carotid sinus massage (34-36) or administration of vagotonic agents (37,38) decrease the frequency of ventricular premature beats and abolish ventricular tachycardia. Since digitalis drugs enhance vagus nerve activity on the heart, we have employed this action of the cardiac glycosides to suppress ventricular arrhythmias (30). This clinical area of endeavor invites further exploration.

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MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF THE MYOCARDIUM AND ITS INNERVATION IN ALCOHOL CARDIOMYOPATHY

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ABSTRACT. A combined morphological, histochemical, biochemical, and electron-microscopic study was performed on 50 persons with alcohol cardiomyopathy (ACMP) who died suddenly. The control group consisted of 18 cases of motor vehicle accident death and 50 cases of sudden cardiac death (SCD) in which there were indications of coronary heart disease (CHD) but no indications of alcohol abuse. Extensive areas of damage to the heart were found in cases with ACMP, with no phosphorylase activity indicated. Diffuse changes in succinate dehydrogenase (SDH) activity were found in the form of granular formazan deposits. Highly specific for ACMP were ultrastructural disorders in the sarcoplasmic reticulum with the formation of cisternae apparently containing the residues of cardiomyocyte organelles. Study of the innervation of the heart showed that both cholinergic and adrenergic plexuses were damaged. Extensive depletion of catecholamines was found in the atria and ventricles. Biochemically, in contrast to cases of SCD with CHD, the concentration of epinephrine in the heart in cases of ACMP was greater than that of norepinephrine. In the hypothalamus, as the central regulator of autonomic peripheral innervation, a pattern of edema of the conduction zone and microcirculation disorders with stases, hemorrhages, and foci of encephalolysis were observed. Cardiac innervation disorders with damage to the correlating centers obviously increased the severity of morphological and functional damage to the myocardium in cases with ACMP.

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INTRODUCTION

Data on the morphological changes in the heart in cases of alcohol cardiomyopathy and the possible interrelationship between these changes and sudden death with this pathology were discussed at the USA-USSR First Joint Symposium on Sudden Death (1). The present study is a continuation of that work and includes a number of new morphological, histochemical, and biochemical parameters.

MATERIALS AND METHODS

In the present study, we examined 50 cases of SCD in persons (48 men and 2 women) from 25 to 55 years of age (with a mean age of 40 years), who were diagnosed as having alcohol cardiomyopathy based on the morphological criteria which we presented at the USA-USSR first joint symposium (1). The control used was 18 persons of the same age who died from motor vehicle accidents, and 50 cases of SCD with signs of CHD in men who did not abuse alcohol.

Twenty-one specimens were taken from various parts of the heart, including its conduction system, according to a special method developed in the Department of Human Cardiovascular Pathology, National Cardiology Research Center, USSR Academy of Medical Sciences (see the paper by Vikhert et al. included in this proceedings). Paraffin sections were stained with hematoxylin-eosin, Masson's stain, and Rego's stain. In nine cases, cryostat sections were used to determine the adrenergic nerve elements, according to Falck's method with various modifications, and the cholinergic elements, according to the method of Karnovsky-Roots. The density of nerve plexus was calculated by the point method (2).

Electron microscope studies were performed on four men within 3 hours after death; specimens of the myocardium were taken from the atria, ventricles, and septa of the heart, fixed in glutaraldehyde, and then treated according to the usual methods. The control used was the heart of intact rats, sections from which were taken before death, and also 15, 30, 60, and 180 minutes after death, as well as the myocardium of 10 men of the same age following sudden death with signs of CHD but with no indications of alcohol abuse.

Histochemical studies of phosphorylase and SDH were performed in seven cases equally soon after death. In parallel with these histochemical studies, we used the same sections of the myocardium in the seven cases to determine biochemically the content of norepinephrine, epinephrine, and their precursor DOPA, using the specific trioxyindol method (3). Morphological and chemical study of the hypothalamus was performed in 10 cases of paraffin sections stained with Nissl's stain using cresyl violet and toluidine blue at pH 5.6, Schiff's reagent, and hematoxylin-eosin. The concentration of alcohol in the blood and urine of the corpses was determined using a type KhL-6 gas chromatograph.

RESULTS

Macroscopically, the heart in cases of alcohol cardiomyopathy was always moderately enlarged due to enlargement of both ventricles (with a mean mass of 425 g). A great deal of fatty tissue was found beneath the epicardium (even in cases of general malnutrition), and the heart frequently had a rounded apex and was flaccid. In section, the myocardium looked like clay, with a yellowish tone, dull, and with no visible focal changes. The coronary arteries had no or only slight atherosclerotic changes without stenosis of the lumen.

Histologically, ACMP was characterized by two closely related processes: progressive atrophy of the muscle fibers and successive development of fatty infiltration of the myocardium. The fatty tissue gradually replaced the disappearing muscle fibers. This sequence was indicated by the changes in morphology, with various stages of myocyte atrophy seen in the same heart. At first, small groups of moderately thin, frequently vacuolized muscle fibers were seen, contained in layers of muscle fiber of normal thickness. As the degree of atrophy and number of atrophied fibers increased, seen in longitudinal sections as much thinner fibers, at first in bundles and then in entire layers, the quantity of fatty tissue in the interstices of these sections progressively increases (figure 1). Whereas in cases of alimentary fat deposition in the heart we observed approximately the same picture only in the wall of the right ventricle, here the same picture is seen in the interventricular septum and in the wall of the left ventricle, where the fatty tissue and atrophied fibers appear in all layers of the wall, including beneath the epicardium. This process is quantitatively different from case to case, but is always focal in nature: more or less discrete areas of fatty infiltration

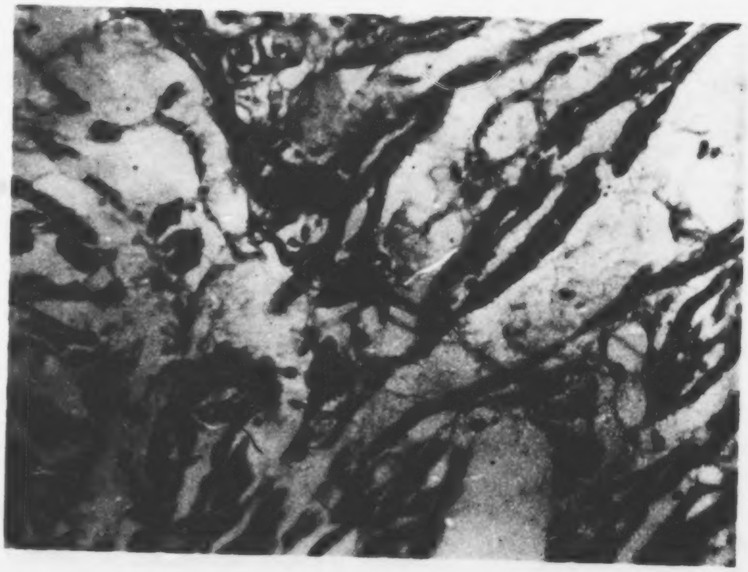

FIGURE 1. Thin, chaotically arranged muscle fibers separated by plates of tissue in sudden death with alcohol cardiomyopathy. Hematoxylin-eosin stain, magnification x 500. Male, 43 years old.

and myocyte atrophy alternate with bundles of moderately enlarged muscle fibers. The latter, in contrast to cases of chronic CHD, appear more monotonic: polymorphism of the muscle fibers themselves and of the nuclei is slight. The nuclei are slightly enlarged and slightly stained.

Finally, a still more extreme stage of the same phenomenon can be observed in the outer layers of both the right and left ventricle walls, where the fatty tissue has almost entirely replaced muscle tissue. Individual cells which look like smooth muscle cells are separated from the muscle tissue, distributed sparsely among the fatty tissue, and oriented in various directions (figure 2). We should add that clear fatty dystrophy is often seen in the atrophied myocytes.

In cases with ACMP, in contrast to cases with CHD, cardiosclerosis is very slight. Fibrous cicatrices replacing muscle tissue are not seen, although in individual cases areas of mild reticular perivascular and subendocardial sclerosis are seen.

When Rego's stain and trichrome Masson's stain are used, we see a quite varied picture of nonuniform localization: scattered, very small or larger lesions in individual muscle fibers; similar multiple lesions over large or limited areas in some sections of the myocardium, alternating with undamaged areas (giving a mosaic pattern); and, finally, solid sections of completely damaged cardiomyocytes of various sizes, sometimes large, and sometimes rather clearly localized in an individual layer of the myocardium on a transverse wall section (figure 3). These damaged areas were most clearly seen in the left ventricle and interventricular septum, and most frequently seen, although sometimes in larger numbers, in the right ventricle; very little damage was observed in the walls of the atrium.

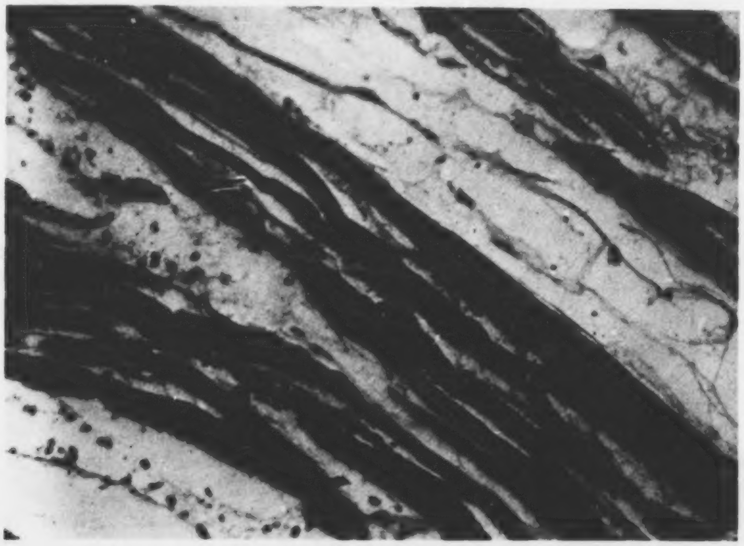

FIGURE 2. Acute degree of atrophy of the muscle fibers in sudden death with alcohol cardiomyopathy. Hematoxylin-eosin stain, magnification x 500. Male, 50 years old.

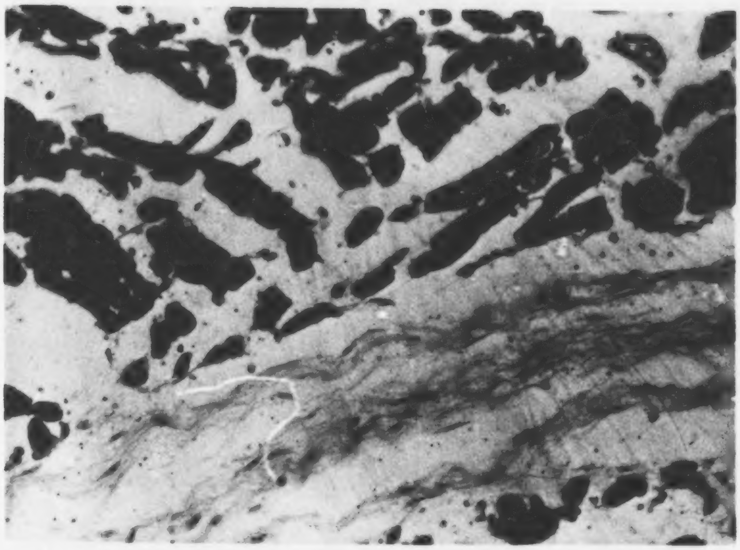

FIGURE 3. Areas of damage in the myocardium. Male, 37 years of age, alcoholic cardiomyopathy. Rego's stain, magnification x 500.

Histochemical determination of phosphorylase activity revealed large, macroscopically visible foci with no stain. No SDH activity in these sections was observed, but it was high and uniform throughout the remaining portion of the preparation. Microscopic examination to determine the distribution of SDH showed that there were individual muscle fibers or groups of fibers in which altered diformazan precipitated in the form of large irregular granules, frequently filling the sarcoplasm, thus indicating the presence of dystrophic reversible or irreversible damage to the myocardium (figure 4).

Study of the cholinergic innervation of the heart in cases of ACMP showed that, in contrast to cases of death from accidents, there was a significant drop in acetylcholinesterase activity in the perikaryons of the nerve cells in the intracardiac ganglia (figure 5). This was never observed in cases of SCD with CHD and without alcohol. Study of the adrenergic innervation showed that regular changes in the adrenergic nerve plexus of the myocardium are practically the same as those described by Shvalev et al. (4) in the myocardium of persons who died suddenly with CHD but without alcohol cardiopathy. These changes included areas of disappearance of specific norepinephrine luminescence in both the atria and the ventricles (figures 6 and 7). The distribution density of adrenergic fibers was significantly lower than in the case of trauma, where it was 7-10 percent, and it was not different from cases of SCD with CHD, where it ranged from 0.1 to 0.4 percent.

Biochemical studies of the catecholamine content in the myocardium showed that the mean content of catecholamines (in ng/g of tissue) for the myocardium as a whole was: epinephrine - 124.8, norepinephrine - 103.4, and DOPA - 205.6.

FIGURE 4. Disrupted succinate dehydrogenase activity in the myocardium of a 43-year-old male with alcohol cardiomyopathy. Magnification x 600.

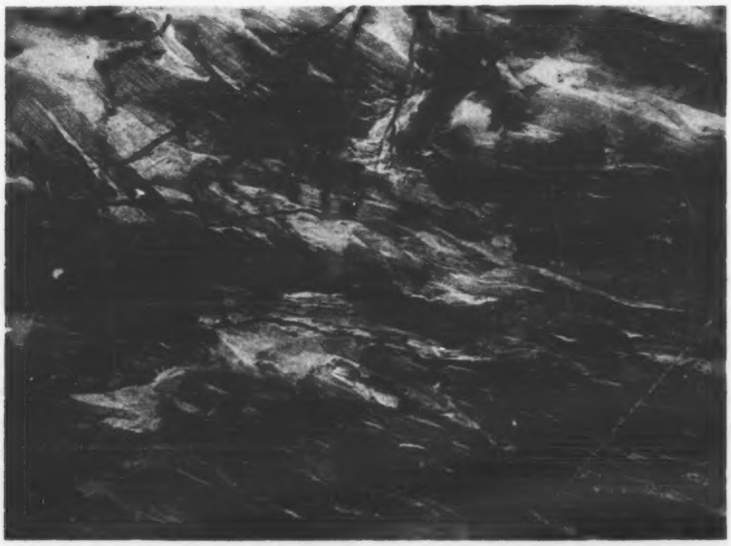

FIGURE 5. Condition of the cholinergic nerve plexus in the right atrium of a male, 47 years old, with alcohol cardiomyopathy. Karnovsky-Roots method, magnification x 222.

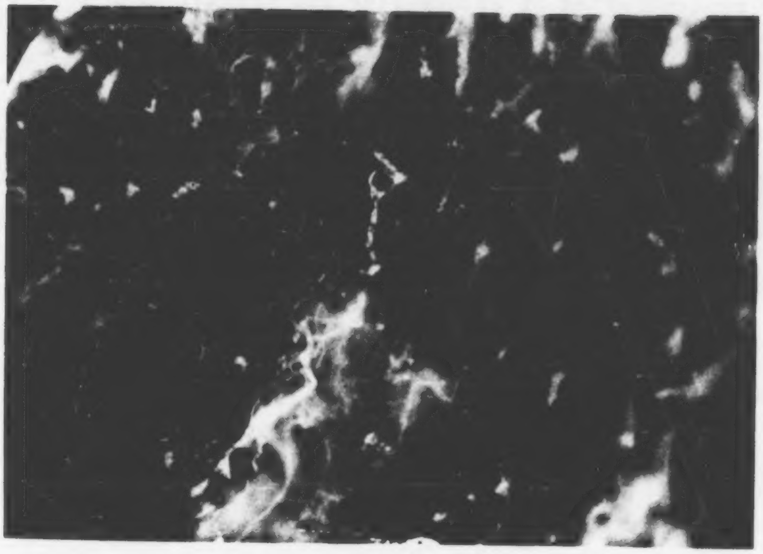

FIGURE 6. Fragment of a preserved adrenergic nerve plexus in the atrium of a male, 43 years old, with alcohol cardiomyopathy. Incubation in 2 percent glyoxylic acid. Gomal 3 stain. Magnification x 40.

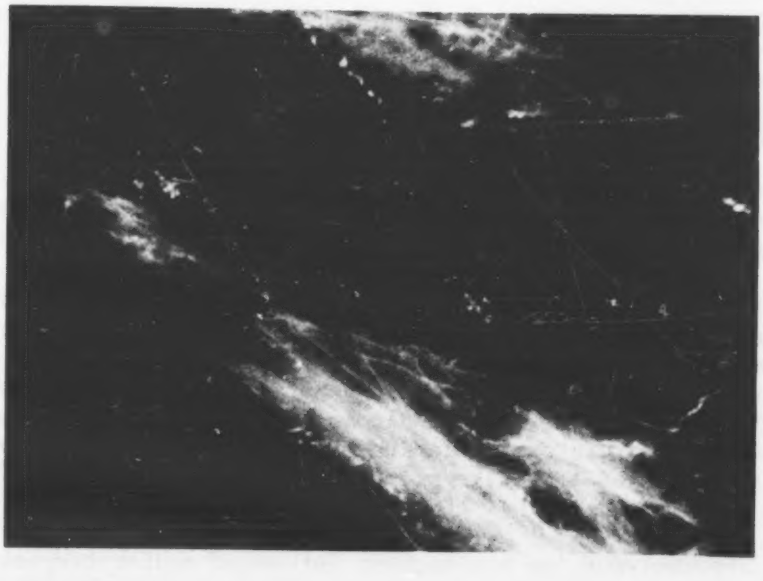

FIGURE 7. Change in the adrenergic nerve plexus of the left ventricle of the heart of a male, 43 years old. Same method and magnification as in figure 6.

That is, the concentration of epinephrine was greater than that of norepinephrine, whereas in cases of SCD without recent foci of infarction, the ratio was the opposite.

In order to determine the capabilities of electron microscopy for investigation of the human myocardium at autopsy and to delineate between autolytic myocardial damage and changes occurring in life, we studied the myocardium of rats after decapitation and primary heart stoppage by intravenous administration of novocaine 15, 30, 60, and 180 minutes after death. This study showed significant stability of the ultrastructure of the cardiomyocytes against autolysis and the feasibility of studying them up to 3 hours after death.

Ultrastructural study of autopsy materials showed that, in cases of ACMP, the changes are more clearly manifest than in persons who died suddenly with indications of CHD. When there were practically intact cardiomyocytes, cells with signs of so-called inconsistent hypertrophy predominated (5). Acute hyperplasia of the small mitochondria and atrophy of the contractile apparatus were observed. In these cells we observed accumulation of lipid inclusions, which is probably a result of disorders in the utilization of fatty acids (6).

Characteristic for cases with ACMP was the presence in the cardiomyocytes of a large number of lysosomes and lysosome-like bodies, as well as lipofuchsin. These changes were seen most frequently in cells with mitochondrial hyperplasia and atrophic myofibrillar apparatus. In cells with preserved ultrastructure, intercrystalline thickening was observed in the mitochondria (figure 8), as described earlier in cases with alcohol cardiomyopathy (7).

One specific indication of ACMP which we discovered is expansion of the cisternae in the sarcoplasmic reticulum with formation of vacuoles containing cardiomyocyte organelle fragments (figure 9). The lysosomes are in close contact with the vacuoles. This change is apparently related to severe disorders in ionic balance, primarily potassium imbalance, which occur with alcohol cardiopathy (8).

The interstitial space was edematous, as a result of which the lumen of the capillaries was significantly decreased; this might lead to disorders in microcirculation, thus aggravating cardiac insufficiency.

Histochemical, histological, electron microscopic, and biochemical studies of the myocardium in cases of ACMP indicated that, in spite of the fact that there are definite morphological indications specific for ACMP, the changes in the nerve apparatus of the heart are in general identical to those observed in cases of SCD related to CHD without alcohol cardiopathy. The same is observed in studies of the centers of regulation of the cardiovascular system, thus allowing us to speak of a unidirectional structure of the changes which primarily vary quantitatively.

In the hypothalamus, as the highest autonomic center responsible for peripheral innervation and innervation of the myocardium in particular, edema predominates in cases of ACMP (figure 10), encompassing not only the white matter, but also the nerve cells themselves; this pattern is not observed in cases of SCD without alcohol intoxication. Severe and diffuse changes of the

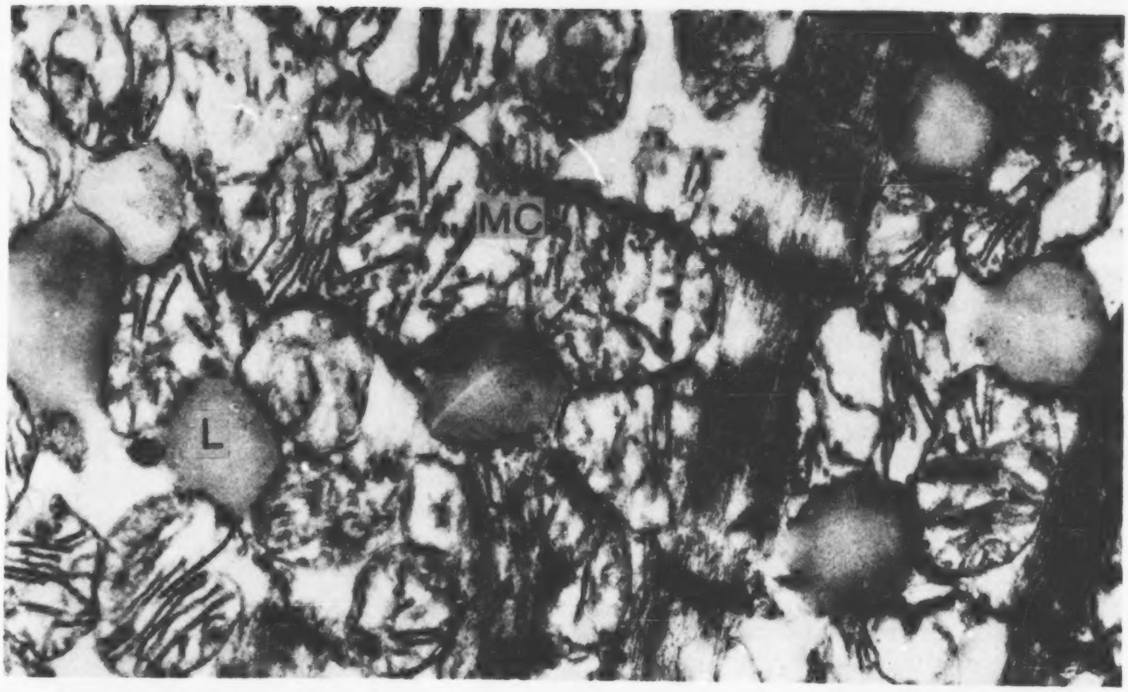

FIGURE 8. Cardiomyocytes from the myocardium of the interventricular septum of a male 43 years old with alcohol cardiomyopathy who died suddenly. Magnification x 25,000. L = Lipid inclusions. MC = Intercrystalline thickening in the mitochondria.

vessels of the hypothalamus are observed: Paretic expansion of the microcirculatory bed is seen everywhere; plasmic saturation of the walls of the arterioles and venules is seen frequently (figure 11); and many small and large hemorrhages are observed in the tissue of the hypothalamus. Many neurons are swollen, with vacuole degeneration. Their nuclei are frequently unclear and pyknotic. In the myelin trunks (in the arch, the optic tract, and the optic chasm) one can frequently see a clear picture of myelin damage with demyelinization; this is never observed in cases of SCD without alcohol.

DISCUSSION

The present study has significantly supplemented our knowledge on alcohol cardiomyopathy and has enabled us to draw certain conclusions. First, we must note that we have observed daily abuse of alcohol for many decades; nevertheless, some of these chronic alcoholics have died at a very advanced age of various causes not related to cardiac abnormalities. The concentration of alcohol in the blood and urine of cases with ACMP rarely exceeds 2.5-3 percent. Furthermore, alcohol may be totally absent although it is reported that the deceased was drinking 1 or 2 days before death. Thus, the presence

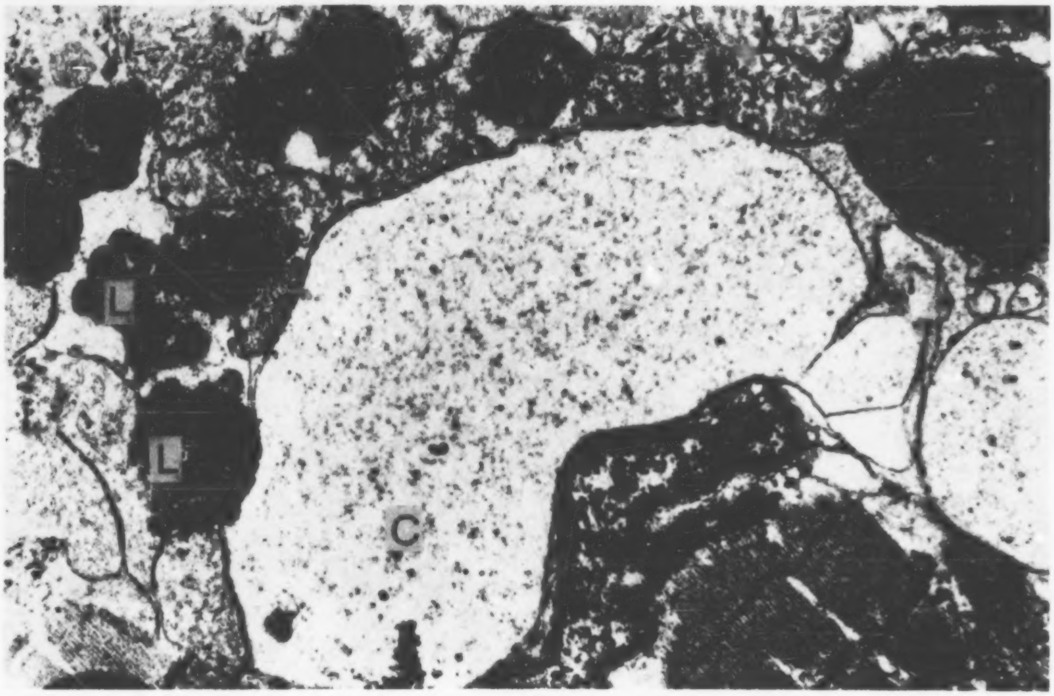

FIGURE 9. Cardiomyocyte from the myocardium of the left ventricle of a male 46 years old. Diagnosis and magnification are the same as in figure 8. C = Expanded cisternae of the sarcoplasmic reticulum. L = Lysosomal structures.

of alcohol at the moment of death cannot serve as reliable evidence of alcohol cardiomyopathy.

In the hearts of cases with ACMP, we see extensive areas of damage, specifically stained with Rego's stain. At the same time, there are areas in various sections of the myocardium that do not show phosphorylase activity. Topographically, these areas do not coincide with the lesions determined by Rego's stain. Outside these areas, we see altered SDH activity, which apparently reflects metabolic problems in the myocardium. Changes in the sarcoplasmic reticulum, consisting of severe expansion with the formation of cisternae containing cardiomyocyte organelle fragments, are quite specific for cases of ACMP. Electron microscope examination revealed certain changes that are characteristic for cases with ACMP; these encompass not only the working cells, but also the microcirculatory bed. Ultrastructural damage is considerably greater in cases with ACMP than in cases of SCD with CHD.

Studies of the innervation of the heart have shown that, in cases with ACMP, both the adrenergic plexus and, in contrast to cases with SCD, the cholinergic plexus are damaged. Catecholamine content in the atria and ventricles is widely depleted, which may result from an increase in the sensitivity

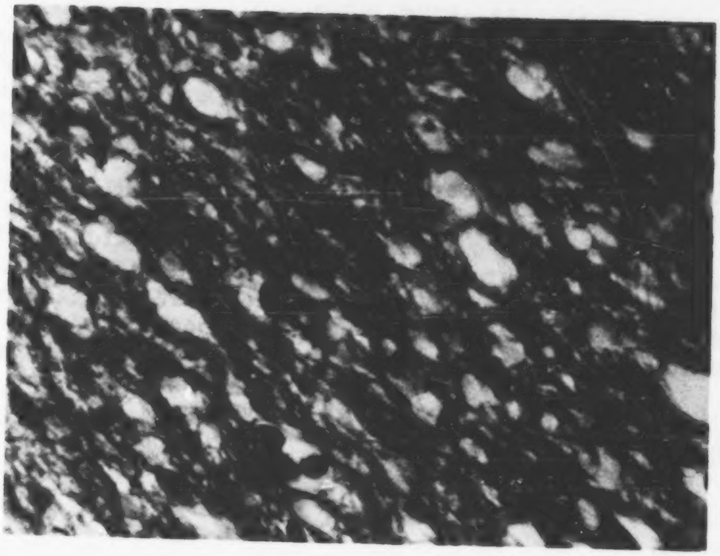

FIGURE 10. Edema of the white matter of the hypothalamus in a male 46 years old who died suddenly with alcohol cardiomyopathy. Hematoxylin-eosin stain, magnification x 380.

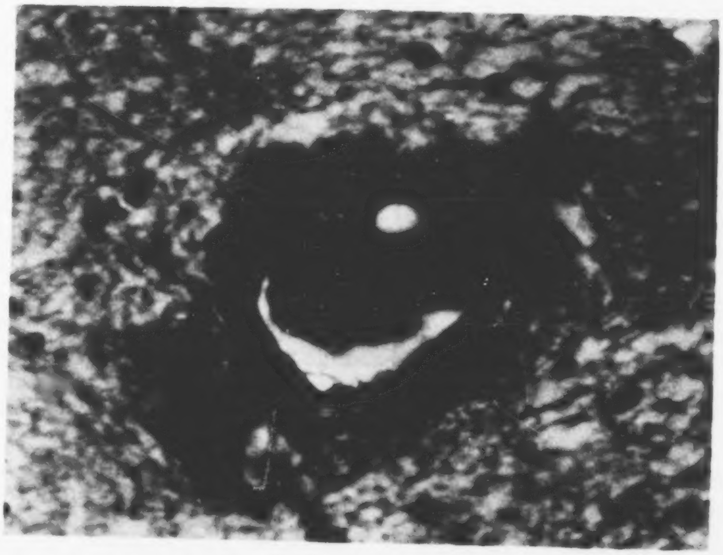

FIGURE 11. Plasmic saturated wall of an arteriole in a male 46 years old. Diagnosis is the same as in figure 10. Schiff stain, magnification x 700.

of the cardiomyocytes to the effect of peripheral catecholamines. This increase in sensitivity is significant because alcohol causes an increase in the concentration of catecholamines in the blood (9,10), which may aggravate the development of damage in the heart muscle.

Disorders in the area of the brain which regulates the cardiovascular system may aggravate stable vascular insufficiency with loss of the main principle of regulation--centralization of blood flow, i.e., conservation of blood flow in vitally important centers (the brain, heart, and kidneys). A possible additional factor is hypoxia which, in addition to the changes noted in the heart, places an added load on the myocardium with which it cannot cope due to its own severe structural changes. To a great extent, this is the cause for the development of acute cardiac insufficiency, heart stoppage, and death.

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REGULATION OF SUBCELLULAR CALCIUM POOLS: MEMBRANE BASES FOR CARDIAC ARRHYTHMIAS

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and August M. Watanabe

ABSTRACT. The myocardium has two well-developed membrane systems responsible for maintenance of ionic gradients, sarcolemma (Sl) and sarcoplasmic reticulum (SR), whose functions are regulated by autonomic neurotransmitters. Whereas the final common pathway in sudden death appears to be ventricular fibrillation, an event ultimately based in Sl, the SR may also contribute to sudden death by controlling intracellular Ca^{2+} concentrations. An in vitro approach was used to characterize Ca^{2+} regulatory components of Sl and SR. Separate subpopulations of Sl and SR membrane vesicles were prepared from canine myocardium. Relative purity of these vesicles was assessed by enzyme marker analysis. Na^+, K^+-ATPase, autonomic receptors, adenylate cyclase, and sialic acid copurified with Sl and were not found in significant quantity in SR. Ca^{2+}, K^+-ATPase copurified with SR, which had negligible levels of Sl protein markers. In intact hearts and in subcellular fractions phosphorylation of distinct Sl and SR proteins was mediated by sympathetic drugs. Experiments are under way to examine the possibility that phosphorylation of integral membrane proteins of Sl may be involved in the catecholamine-produced augmentation of slow inward current, whereas phosphorylation of SR proteins may affect components involved in Ca^{2+} uptake and Ca^{2+} release. Thus, phosphoproteins of both Sl and SR may regulate intracellular Ca^{2+} concentrations. This, in turn, could affect ionic permeabilities of Sl, resulting in disorders in both automaticity and conduction.

From the Department of Pharmacology and Toxicology, the Department of Medicine, and the Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, Indiana. This work was supported by grants S01-RR-95371, HL-23704, HL-06308, HL-18795, and HL-07182 from the National Institutes of Health; by the American Heart Association, Indiana Affiliate; by the Herman C. Krannert Fund; and by the Showalter Residency Trust.

INTRODUCTION

Since the basis of electrical activity in the myocardium is the asymmetric distribution of ions across membranes, alterations of ion handling by myocardial membranes must ultimately contribute to sudden cardiac death. The myocardium has two well-developed membrane systems responsible for maintenance of high ionic gradients. These are the external plasma membrane and its invaginations (or sarcolemma) and the internal system of tubular membranes (the sarcoplasmic reticulum). It is well-established that calcium ions affect the electrical performance of myocardium and that both Sl and SR participate in regulation of myocardial calcium ion activities. Furthermore, catecholamines have been shown to exert actions on both the Sl and the SR (1), and recent evidence shows that acetylcholine may alter the myocardial cellular perception of sympathetic neurotransmitter signals (2).

The long-term objective of our studies has been to determine the molecular bases of the effects of catecholamines and of acetylcholine on cardiac membrane functions. These studies were begun with the major focus on calcium handling by these two membrane systems. During the course of our investigations we have attempted to separate and purify Sl and SR, the two major calcium handling membranes; to identify and characterize the slow inward calcium current channel, at the molecular level; and to determine the molecular basis of the functional interaction of adrenergic with cholinergic stimulation of myocardial membranes. The present report reviews experimental progress made toward understanding the first two goals above.

METHODS

Studies on isolated membrane fractions were done with impure microsomal fractions from canine myocardium (3) as well as with purified preparations of Sl and SR in the form of vesicles (4). Phosphorylation of membrane proteins in intact hearts was done by a technique similar to that recently reported by Kopp and Barany (5).

RESULTS AND DISCUSSION

Although cardiac microsomes contain a mixture of membrane vesicles formed from several cellular membranes, enzyme marker analysis demonstrates that the combined percentage of SR and Sl vesicles represent about 80-90 percent of the total. Subpopulations of crude vesicles fractionated on sucrose density gradients show patterns of enzymic and nonenzymic markers that demonstrate a high degree of purification of the microsomal vesicles into subpopulations of Sl and SR (figure 1). Thus, beta-adrenergic receptor sites, adenylate cyclase, and sialic acid all comigrate with the sarcolemmal marker enzyme Na$^+$, K$^+$-ATPase in sucrose gradient fractions. On the other hand, the SR marker enzyme Ca^{2+}, K$^+$-ATPase distinctly separates from the sarcolemmal enzymes.

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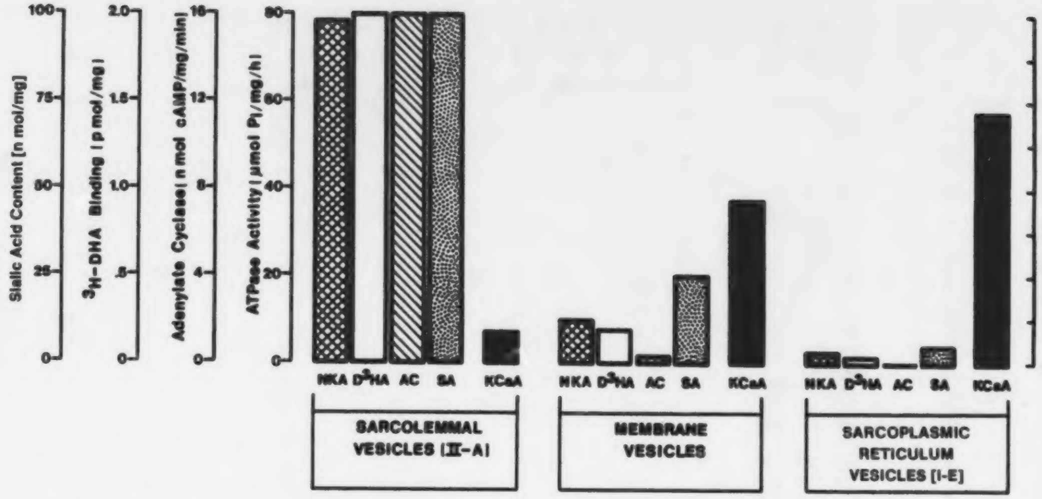

FIGURE 1. Comparative biochemical activities of relatively purified sarcolemma (II-A), sarcoplasmic reticulum (I-E), and crude cardiac membrane vesicles. The activities assayed were: Na^+, K^+-ATPase (NKA); [^3H] dihydroalprenolol binding (D^3HA); adenylate cyclase (AC); sialic acid (SA); and K^+, Ca^{2+}-ATPase (KCaA).

Consistent with the complex functions of S1 and SR, the protein composition of these purified membranes revealed no fewer than about 30 separable components in each. Polyacrylamide gel electrophorograms of the S1 and SR subfractions of cardiac microsomes, compared with the crude parent membrane vesicle fraction, are presented in figure 2. Comparisons of the staining profiles utilizing three separate dyes (Coomassie blue, periodic acid-Schiff stain, and Stains-All) show that at least five of the major proteins present in the sarcolemmal fractions reside exclusively in this membrane. Similarly, at least eight distinct proteins are intrinsic to SR only.

Much evidence exists to show that several principal effects of catecholamines are initiated by cyclic adenosine 3':5'-monophosphate (cyclic AMP)-stimulated phosphorylation reactions (1). In initial attempts to localize the membrane protein substrates of cyclic AMP-dependent protein kinases, we used in vitro preparations of cardiac S1 and SR (4). Purified S1 and SR were incubated with [γ-^{32}P]ATP (adenosine triphosphate) both in the absence and presence of cyclic AMP. For the S1 preparation, cyclic AMP stimulated [^{32}P] incorporation into at least six protein bands of molecular weights ranging from 200,000 to 8,000 (figure 3). Presumably, these proteins were substrates of an intrinsic cyclic AMP-dependent protein kinase activity of S1. The regulatory subunit of the protein kinase is the dark band with a molecular weight of approximately 55,000 (unpublished observations). It is reasonable to suspect that either of the protein kinase substrates of about 10,000 molecular weight or 8,000 molecular weight may be involved in the regulation of the slow inward calcium current (1,4). As shown in the right panel of figure 3, SR proteins were also phosphorylated in the presence of [γ-^{32}P]ATP. However, this [^{32}P] incorporation

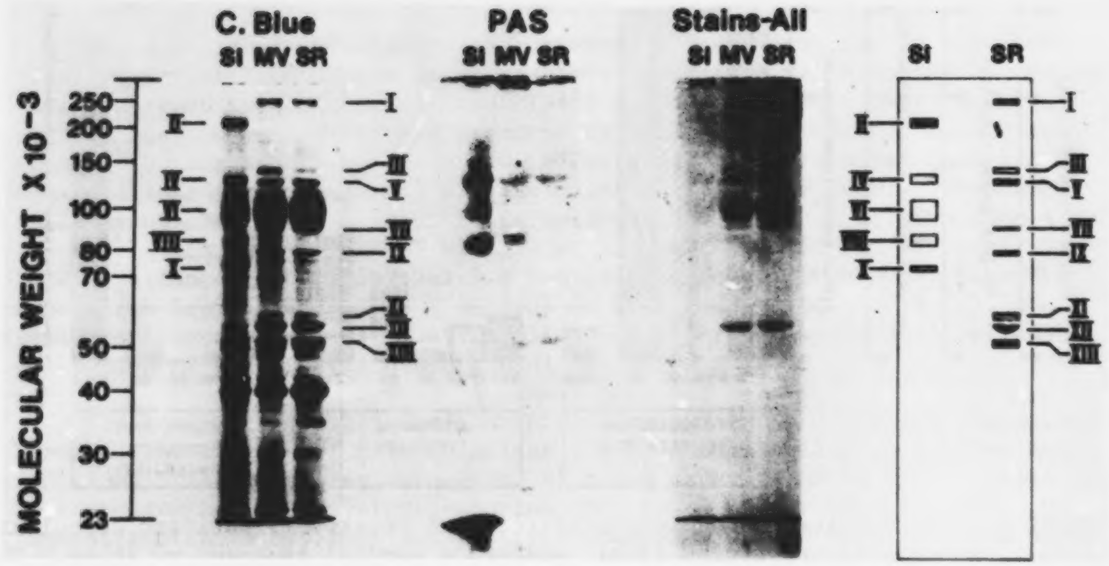

FIGURE 2. Polyacrylamide gel electrophoresis of membrane vesicle fractions solubilized in sodium dodecyl sulfate. Sl = Sarcolemma. MV = Membrane vesicle. SR = Sarcoplasmic reticulum. All samples were electrophoresed in the same slab of polyacrylamide (8.5 percent). After electrophoresis, the gel was cut into three sections and each section was stained with either Coomassie blue (C. Blue), periodic acid-Schiff reagent (PAS), or Stains-All. Twenty-five micrograms of protein were applied per well when staining with C. Blue or Stains-All and 80 μg of protein were applied per well when staining with PAS. Apparent molecular weights of the designated bands (± 5 percent), estimated from several runs of membrane samples and protein standards with several concentrations of acrylamide (7, 8.5, and 10 percent), were as follows (x 10^{-3}): I-265; II-210; III-142; IV-135; V-127; VI-100; VII-90; VIII-85; IX-80; X-75; XI-59; XII-55; and XIII-51. Bands were arbitrarily numbered in reference to the section stained with C. Blue for comparison of profiles between the three stains. (Reprinted with permission from reference 4)

was not stimulated by cyclic AMP. If anything, cyclic AMP slightly diminished the total phosphate incorporation, as shown by the shaded bars. We can conclude, therefore, that SR does not contain an intrinsic cyclic AMP-dependent protein kinase. It has been shown by others (1) and ourselves (4), however, that SR does contain protein substrates of soluble, cytoplasmic cyclic AMP-dependent kinase. One of these is phospholamban (6).

It is pertinent to ask whether experiments conducted in vitro on isolated membranes have any relevance to what actually might be occurring in vivo. In an attempt to address this question, we labeled the endogenous phosphate pools of isolated, perfused guinea pig hearts with radioactive inorganic phosphorous ([^{32}P]). After an equilibration period, it can be demonstrated that the

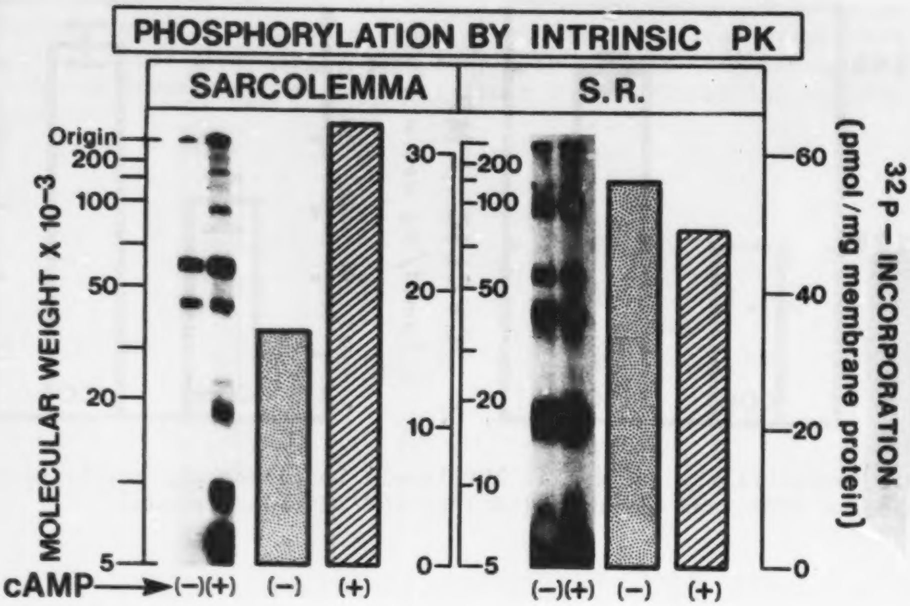

FIGURE 3. Phosphorylation produced in sarcolemmal and sarcoplasmic reticulum (S.R.) fractions by intrinsic cyclic AMP-dependent protein kinase (PK). Phosphorylation was carried out for 1 minute at 23° C in a medium containing 0.1 mg/ml membrane protein 21 µM [γ-^{32}P]ATP, 50 mM histidine, 3 mM $MgCl_2$, 10 mM potassium fluoride, 1 mM 3-isobutyl, 1-methyl xanthine, 1 mM Tris/EGTA, and 100 mM KCl, with or without 10 µM cyclic AMP. Polyacrylamide gel electrophorograms were prepared after extensive washing of the membranous material in 5 percent trichloroacetic acid, 20 mM NaH_2PO_4, and 2 mM Na_2ATP. The bars represent values determined by counting aliquots of the washed phosphorylated membranes before polyacrylamide gel electrophoresis.

metabolically active heart cells have converted a fraction of [^{32}P] to [γ-^{32}P]ATP (5). Isoproterenol (at a concentration of 10^{-7} M) was then added to the perfusion fluid and changes in contractility, cellular cyclic AMP levels, and incorporation of [^{32}P] into cardiac microsomes were measured. Figure 4 shows the increase in contractility (dT/dt) and the corresponding increase in cellular cyclic AMP levels within the hearts. It is clear that both increased, as expected in hearts perfused with isoproterenol.

After 2 minutes of perfusion, the hearts were quickly freeze-clamped and membrane vesicles were prepared from these hearts. Gel electrophoresis and autoradiography of the solubilized membranes was done to evaluate if membrane proteins had been phosphorylated in intact, functioning heart. Following adrenergic stimulation with isoproterenol, there was increased specific phosphorylation of at least two membrane proteins (figure 5). These proteins migrated with apparent molecular weights of about 20,000 and 10,000, and may correspond to proteins of similar mobility which can be phosphorylated in purified membrane preparations in vitro.

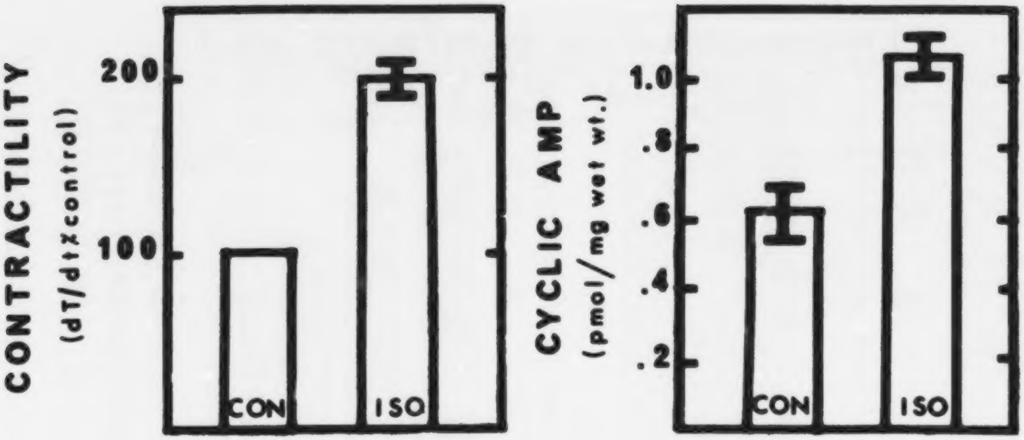

FIGURE 4. Contractility and cyclic AMP levels in guinea pig hearts perfused in the absence (CON) and presence (ISO) of 10^{-7} M isoproterenol.

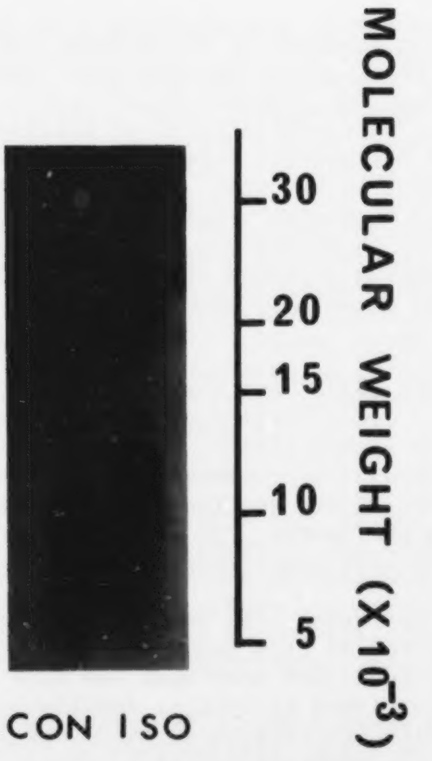
MOLECULAR WEIGHT (X 10^{-3})
30
20
15
10
5
CON ISO

FIGURE 5. Autoradiograph of membrane proteins phosphorylated in the absence (CON) and presence (ISO) of isoproterenol (10^{-7} M) in intact, functioning guinea pig ventricles.

Thus, it may be possible that membrane protein phosphorylation is the final pathway by which autonomic drugs alter the electrical properties of the heart. Since these phosphorylations result in altered Ca^{2+} metabolism by membrane-bound proteins, they may contribute to electrical instability of the sarcolemma.

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DYNAMICS OF FUNCTIONAL AND ULTRASTRUCTURAL CHANGES IN THE MYOCARDIUM AT VARIOUS TIMES AFTER ADMINISTRATION OF RESERPINE

N. N. Beskrovnova, L. S. Ulyaninsky, V. A. Makarychev, and A. M. Vikhert

ABSTRACT. Experiments with 23 rabbits were conducted to study the dynamics of ultrastructural and electrophysiological disturbances, and their role in the onset of sudden death from ventricular fibrillation after various periods of sympathectomy (up to 24 hours) caused by the administration of reserpine at a dose of 2.5 mg/kg of body weight. The thresholds of excitability of the myocardium and occurrence of ventricular fibrillation were studied using a biophasocardiosynchronizer. Both vagus nerves were sectioned in some of the experiments to exclude the parasympathetic effect. No significant changes in the ultrastructure, other than the accumulation of granules of glycogen in the sarcoplasm of the cardiomyocytes, occurred after 30 minutes of reserpine activity. An even greater increase in the content of glycogen in the cells was observed after longer periods of reserpine activity (1-24 hours) in combination with increasing destructive changes in the organelles in the form of many sites of enhanced contraction of myofibrils, destruction of mitochondria, destruction of the sarcolemma, etc. Morphological changes correlate well with the electrophysiological indices. Ventricular fibrillation thresholds were reduced after 24 hours of sympathectomy, but the fibrillation process was irreversible in the rabbits as compared with the control animals. The study made it possible to establish that sympathectomy caused by the administration of reserpine results initially in disturbance of carbohydrate metabolism with subsequent progressive development of gross destructive changes in the cell organelles. This may be the cause for the reduction in fibrillation thresholds and the onset of sudden death. The reduced fibrillation thresholds in these experiments are independent of the tone of the vagus nerve.

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INTRODUCTION

It is known that the principal cause of sudden death among patients who have a heart attack is ventricular fibrillation. Great importance in the onset of lethal arrhythmias is attached to a change in the function of the autonomic nervous system, and in particular to the activation of its sympathetic section. However, histochemical methods have been helpful in showing that there were signs of depletion of the catecholamine stores in the adrenergic nerve plexuses and ganglia (1,2) in some patients who died suddenly. Because of the contradictory data on the role of the sympathetic nervous system in sudden death, we undertook the present research to study cardiac sensitivity to fibrillation as a function of the condition of its structure against a background of pharmacological sympathectomy. The model of sympathectomy used was one in which the animal was administered reserpine. It is known that reserpine depletes the stores of catecholamines in the body (3).

MATERIALS AND METHODS

The experiments involved the use of 23 chinchilla rabbits. Reserpine (Rausedil - A. O. Gedeon Chemical Plant, Budapest, Hungary) was administered at a dose of 2.5 mg/kg of body weight. This dosage was selected based on our own preliminary experiments and on data in the literature. A severe sympathectomic effect was observed when this dosage was used. The drug was administered intramuscularly.

The animals were killed 30 minutes, and 1, 4.5, and 24 hours after the drug was injected in order to conduct electron microscopic studies. Sections of the myocardium were taken from the left ventricle wall and from the peripheral Purkinje fibers. The material was fixed in 2.5 percent glutaraldehyde in a 0.1 M phosphate buffer, followed by final fixation in 1 percent osmic acid in the same buffer, dehydrated in increasing concentrations of alcohol, and poured into a mixture of epon and araldite. Ultrathin sections were prepared using a Reichert Om-3 ultratome, contrasted, and viewed in a JEM-100-B electron microscope.

Ventricular fibrillation thresholds were measured by connecting a pulse train in the vulnerable phase of the cardiac cycle in the case of the control animals, and from the effect of the reserpine in the case of the rabbits, at later times (19-24 hours) after the drug was injected. Nembutal anesthesia and a respirator were used to perform the thoracotomy. The pericardium was opened and the electrical pulses were delivered to the right ventricle through a bipolar electrode. A biophasocardiosynchronizer (BFS-1) with a 60-90 msec delay was used to deliver 15-25 square pulses at a frequency of 100 Hz and a duration of 4 msec from the R wave of the electrocardiogram (ECG) to the heart. The ventricular fibrillation threshold was determined while the strength of the effect was increased discretely. The threshold force of the current was measured with respect to the voltage drop across a 1 kΩ resistor connected in series with the electrode taps. We had previously used this test to study the vulnerability of the heart to fibrillation in rabbits (4). The excitability and absolute nonsusceptible period were determined using a point electrode-cathode inserted into the myocardium of the right or left ventricle and by applying a single 5 msec square pulse at various phases of the cardiac cycle. A bilateral cervical vagotomy was performed on some animals during the experiments to avoid the parasympathetic effect.

RESULTS

Thirty minutes after the reserpine was injected, cells with definite ultrastructural changes in the mitochondria and glycogen content were discovered in the myocardium, along with intact cardiomyocytes (figure 1A). An increase in the content of glycogen granules in the cells was the most characteristic feature. Glycogen granules uniformly filled almost all of the sarcoplasm. The mitochondria were swollen, increased in size, and had a nonuniformly packed matrix and few cristae. Some of the cristae were destroyed (figure 1B). No foci of enhanced contraction of myofibrils were seen during this period, in contrast to later periods.

These changes increased after 1 hour, and disturbances in the structure of the contractile elements were added. Numerous glycogen granules appeared in the sarcoplasm of the cardiomyocytes, filling almost all of the sarcoplasm, and often accumulating in the perinuclear zone (figure 2A). The bulk of mitochondria in the cells presented destructive changes. These included swelling of the mitochondria, increase in size, destruction of cristae, and, in a number of cases, destruction of the outer mitochondrial membrane. Multiple lipoprotein complexes were often found near damaged mitochondria. The tubules of the sarcoplasmic reticulum were considerably enlarged and appeared optically clear. Individual focal contractions and enhanced contraction of the myofibrils were revealed in some cardiomyocytes during this period of reserpine effect. Even more pronounced contractile changes in the myofibrils against a background of considerable accumulation of glycogen and destructive changes in the mitochondria were detected at later periods of the reserpine effect: 4.5 and 24 hours after administration of the drug.

Sites of enhanced contraction of the myofibrils were noted in many cells 4.5 hours after the administration of reserpine. The foci of this enhanced contractility appeared to be dense and homogeneous; the parallel location of the actinoid and myosinoid filaments in the myofibrils was disturbed; and the transverse striation characteristic of myofibrils had disappeared. Merging and thickening of the Z discs were noted in the areas of enhanced contractility and tears and destruction of the myofilaments were found (figure 2B).

Numerous areas of excessive contraction of the myofibrils in combination with destructive changes in the mitochondria, and many glycogen granules in the sarcoplasm, were observed in virtually all cells 24 hours after administration of the drug. The glycogen granules were particularly numerous in the perinuclear zone. The mitochondria were distinctly swollen, contained individual cristae, and the structure of individual mitochondria was completely destroyed. The tubules of the sarcoplasmic reticulum were enlarged (figure 3A). Multiple sites of excessive contraction of the myofibrils were found in the cardiomyocytes (figure 3B). The sarcolemma and intracellular organelles in the enhanced contracted cells were often destroyed, and glycogen granules and lipoprotein complexes were found in the extracellular space.

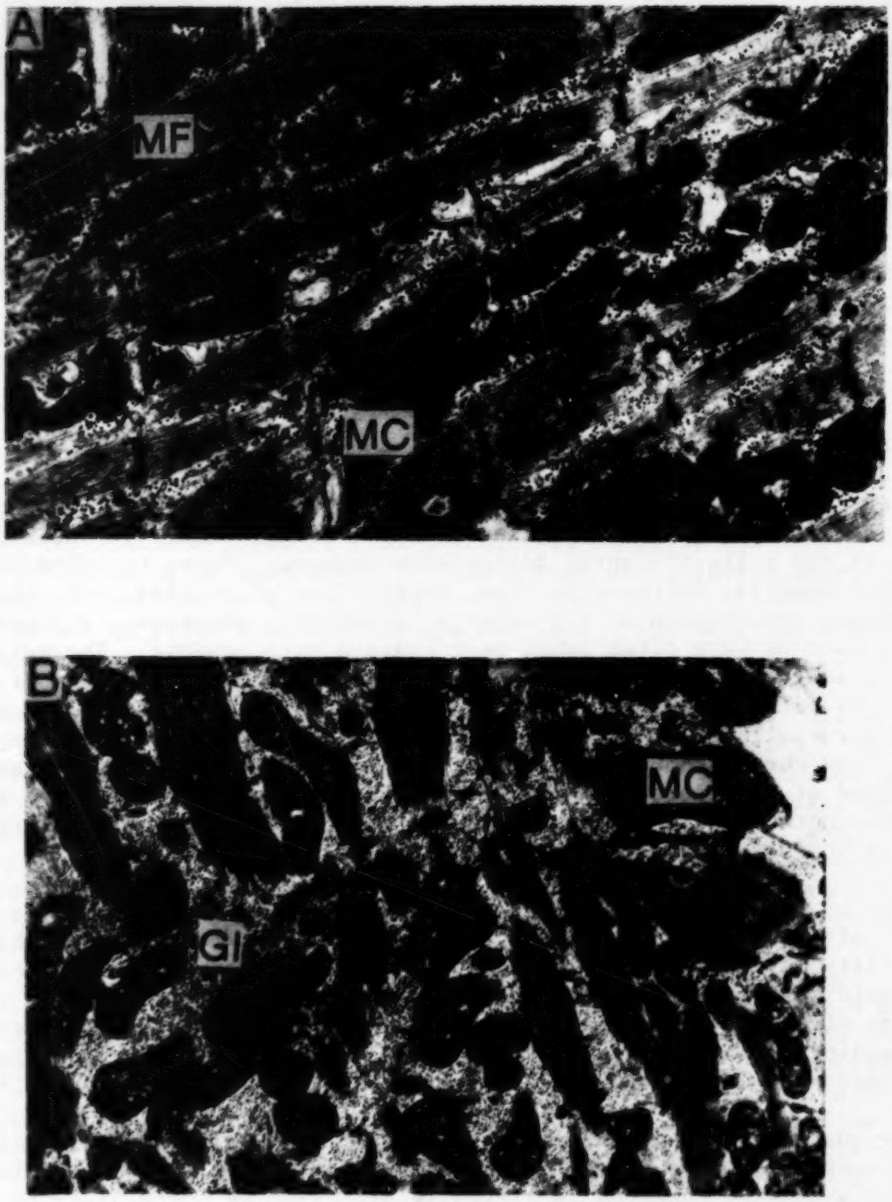

FIGURE 1. Ultrastructure of rabbit myocardium before and 30 minutes after administration of reserpine. A = Cardiomyocyte of control rabbit, magnification x 20,000. B = Accumulation of glycogen granules (Gl) in cell sarcoplasm 30 minutes after reserpine, magnification x 12,000. MF = Myofibril. MC = Mitochondria.

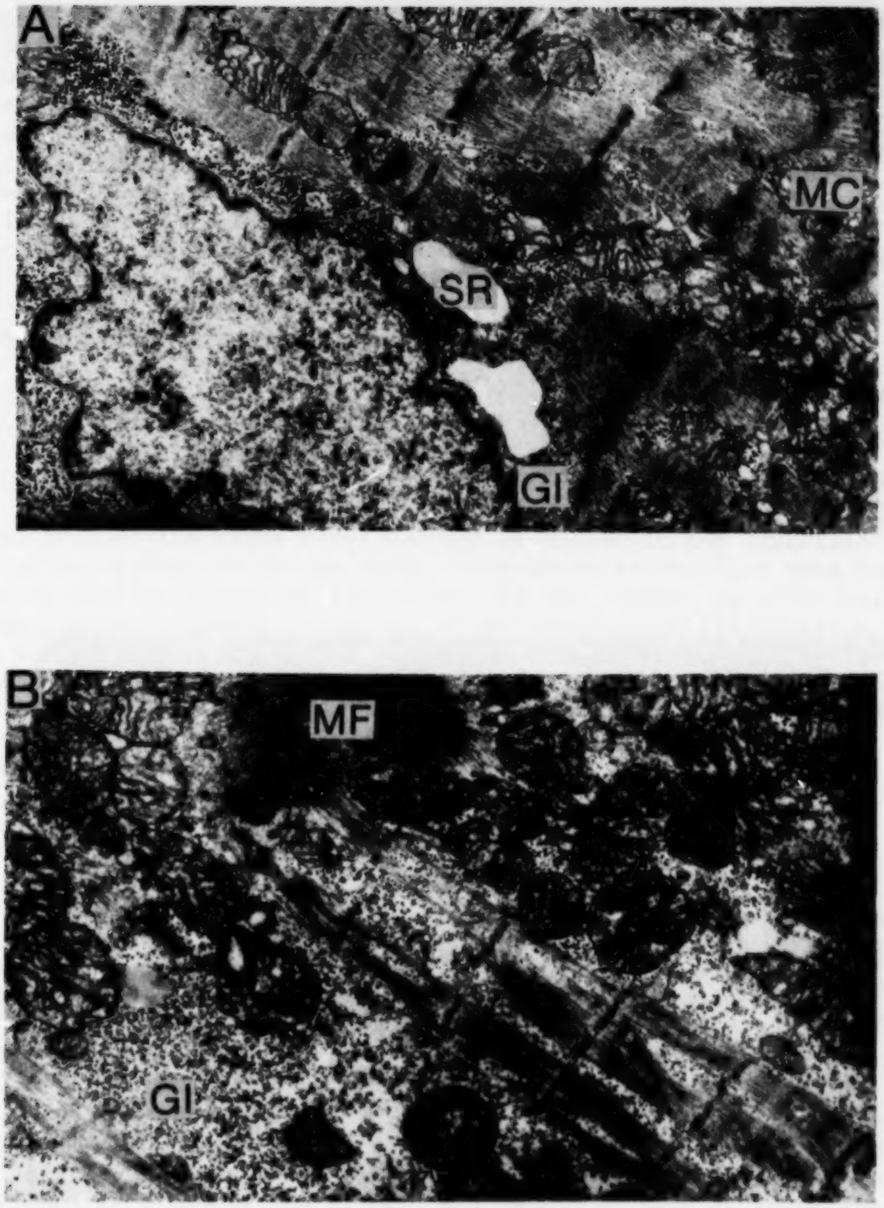

FIGURE 2. Ultrastructure of rabbit myocardium 1 and 4.5 hours after reserpine. A = Perinuclear zone of cardiomyocyte 1 hour after reserpine, magnification x 15,000. B = Focal enhanced contractions of myofibrils (MF) against a background of numerous glycogen granules (Gl) and destruction of mitochondria (MC) 4.5 hours after reserpine, magnification x 15,000. SR = Sarcoplasmic reticulum.

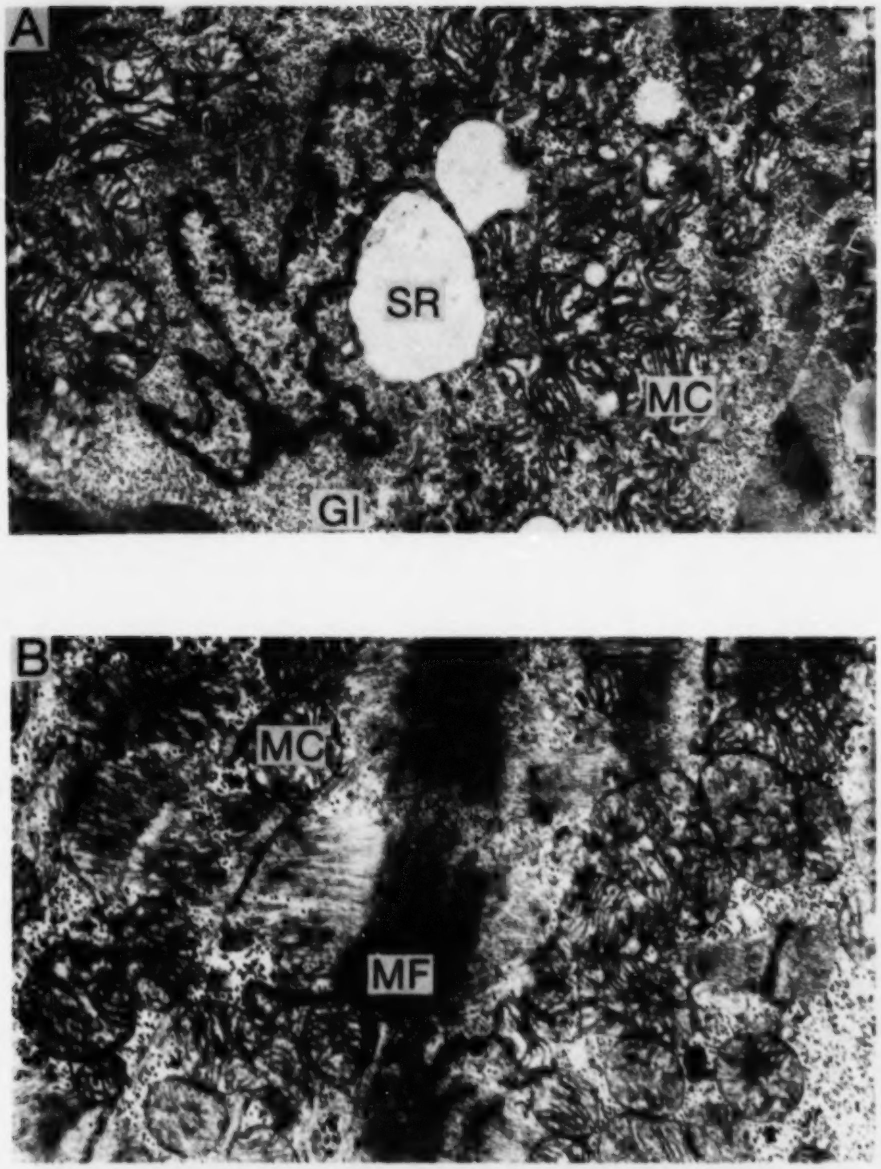

FIGURE 3. Ultrastructure of rabbit myocardium 24 hours after administration of reserpine. A = Accumulation of glycogen granules (Gl), destruction of mitochondria (MC), enlargement of tubules in the sarcoplasmic reticulum (SR) in the perinuclear zone of the cell, magnification x 10,000. B = Contractile changes in the myofibrils (MF), destruction of mitochondria, and accumulation of glycogen granules in the sarcoplasm, magnification x 15,000.

Electron microscopic study of the Purkinje cells at all times of reserpine activity showed that the structure of these cells remained intact as compared with the cardiomyocytes of the working myocardium. The basic mass of Purkinje cells was intact at 30 minutes, 1 hour, and 4.5 hours after administration of the drug, and in no way did these cells differ from identical cells in the control rabbits. Numerous glycogen granules, normally characteristic of these cells were detected in the sarcoplasm of the Purkinje cells. There were no significant changes in the structure of the mitochondria or contractile elements (figures 4A and 4B). Only 24 hours after the administration of reserpine was it possible to observe in some of the Purkinje cells individual areas with enhanced contraction of the myofibrils and swelling of the mitochondria with destruction of the cristae against a background of numerous glycogen granules.

Parallel electrophysiological study revealed that reserpine, at the height of its effect (19-24 hours after administration of the drug), slows the rate of cardiac contractions by approximately a factor of 2 (134 ± 7 beats/min) compared with the initial level in the control animals (250 ± 19 beats/min) (table 1). At the same time, the duration of the absolute refractory period increased, while there was practically no change in the threshold of diastolic excitability.

The tone of the vagus nerve was not developed in the experiments with the effect of reserpine, or in the norm. Indicative is the fact that bradycardia, induced by administration of reserpine, was not cleared up after bilateral vagotomy. A considerable reduction in the ventricular fibrillation threshold in the animals was noted as the reserpine took effect. As in the control group of animals, the onset of fibrillation was observed when the current flow was 18 ± 0.51 mA, and the brief ventricular tachysystole, or fibrillation, occurring during this period was, as a rule, reversible (figure 5). On the other hand, the fibrillation threshold in the experimental group of rabbits fell to 12 ± 0.86 mA, and the fibrillation that did occur was irreversible (figure 6). Defibrillation was required to restore heart rhythm. There were, however, a number of cases of bradycardia or cardiac arrest after defibrillation.

DISCUSSION

This study resulted in the discovery of definite dynamics for disturbances in the ultrastructure of rabbit myocardium under the effect of a 2.5 mg/kg dose of reserpine, and the relationship between these disturbances and electrophysiological changes in the heart. The earliest evidence of the effect of reserpine on the myocardium is the accumulation of glycogen granules in the sarcoplasm of cardiomyocytes. The first signs of an increase in the content of glycogen in the cells were observed early (at 30 minutes) under the effect of the drug, but the tendency to accumulation of glycogen remained, even at later stages of the effect. In these cases, successively increasing destructive changes occurred in the intracellular organelles against the background of increased glycogen content. This increase in the content of glycogen in the myocardium after administration of reserpine agrees with the results of other studies (5-7).

The mechanism of this process is still unclear, but one can suppose that one of the reasons for accumulation of this carbohydrate in the cell could be the sharp reduction in the content of catecholamines in the organism--a result

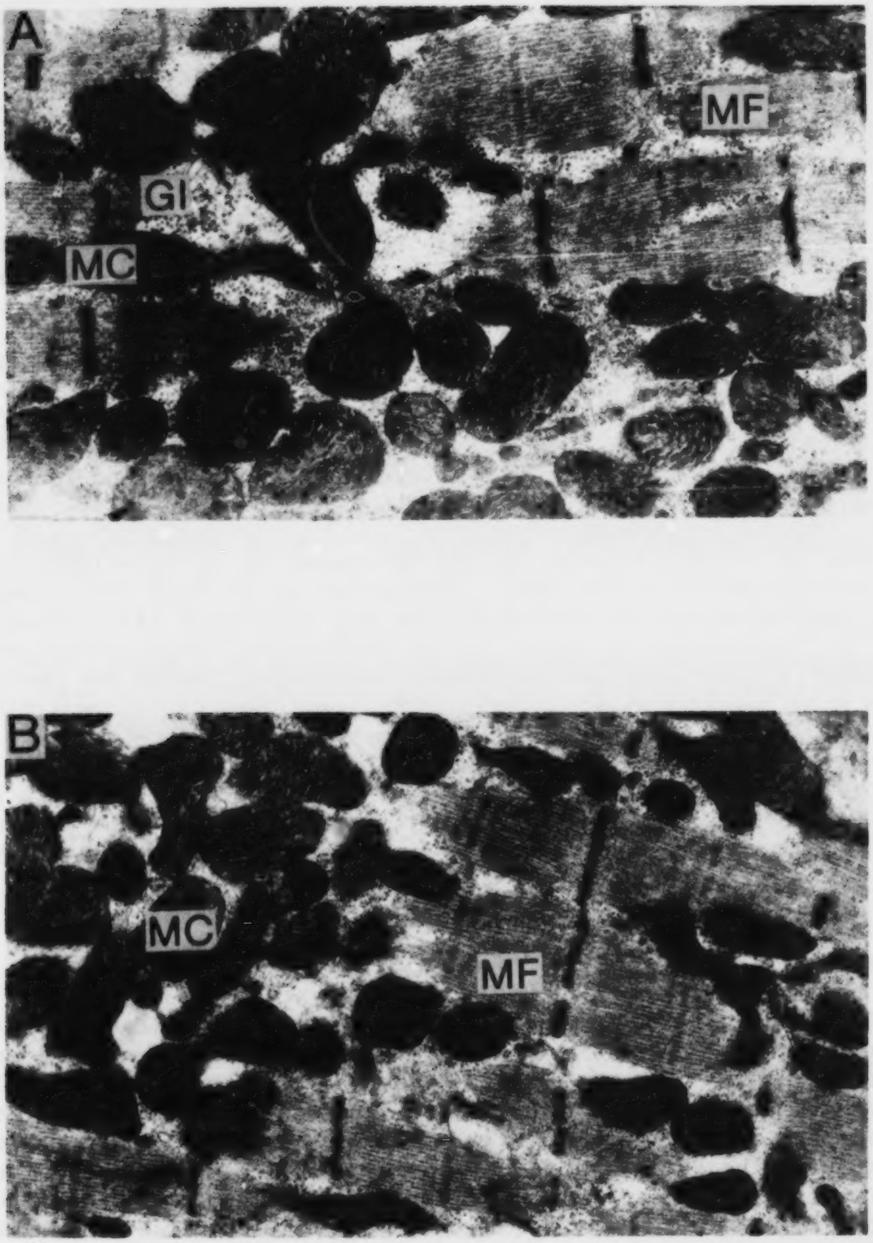

FIGURE 4. Ultrastructure of the Purkinje cells before and after administration of reserpine. A = Fragment of a Purkinje cell from a control rabbit, magnification x 20,000. B = Fragment of a Purkinje cell 4.5 hours after reserpine. Ultrastructure unchanged, magnification x 20,000. MF = Myofibril. MC = Mitochondria. Gl = Glycogen.

TABLE 1. Change in the Frequency of Heart Contractions, Duration of Absolute Refractory Period, Threshold of Diastolic Excitability, and Fibrillation Threshold in Rabbits Under the Effect of Reserpine (M ± m) (Data From 23 Experiments)

Electrophysiological parameters	Control animals	19-24 hours after injection of reserpine, 2.5 mg/kg	p
Frequency of heart contractions per minute	250 ± 19	134 ± 7.00	$p < 0.01$
Duration of absolute refractory period in msec	110 ± 11	150 ± 5.7	$p < 0.01$
Threshold of diastolic excitability in mA	0.45 ± 0.07	0.38 ± 0.05	$p > 0.2$
Threshold of fibrillation in mA	18.3 ± 0.51	12 ± 0.86	$p < 0.01$

of the effect of reserpine. It is known that a definite level of catecholamines is necessary for the functioning of the cell's phosphorylase system. It has been pointed out that, when reserpine is working, one can observe in the myocardium a reduction in the activity of the enzymes of glycogenolysis with rather high glycogen-synthetase activity (6). According to our data, accumulation of glycogen in the myocardium obviously does not inhibit ventricular fibrillation in rabbits treated with reserpine.

Numerous areas of enhanced contraction of myofibrils, destructive changes in the mitochondria and in other cellular organelles, appeared along with an increased glycogen content at later periods in the reserpine effect. The observed disturbances in the structure of the mitochondria may be indicative of a sharp reduction in the energy-forming function of these organelles and, in conjunction with other morphological changes, suggest an energy deficit in the cell. The contractile changes that take place in the myofibrils are irreversible, and have already been observed by other researchers in a variety of situations associated with overloading the cell with calcium ions (8-10), as well as under the effects of a toxic dose of strophanthin on rabbit heart (11).

The reason for such disturbances of the ultrastructure of the myocardium in animals that have been injected with reserpine is still unclear. However, the literature does contain information pointing to the sensitivity of the heart to many effects, and in particular to calcium; that sensitivity is increased after the animal has been injected with reserpine (12-14). Therefore, we cannot exclude the fact that the excessive contractions of the

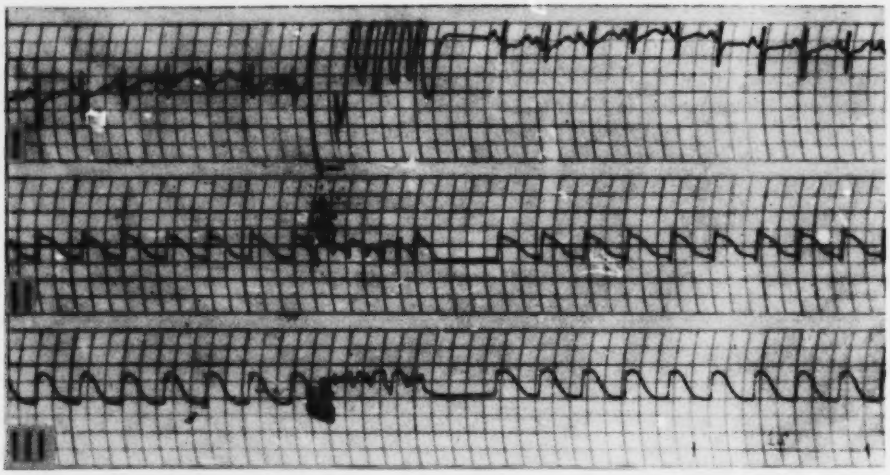

FIGURE 5. Occurrence of momentary ventricular tachysystole in control rabbits in reaction to a 15 mA pulse train applied to the heart. I = ECG recording. II and III = Electrograms of the left and right ventricles, respectively, obtained with sucker electrodes.

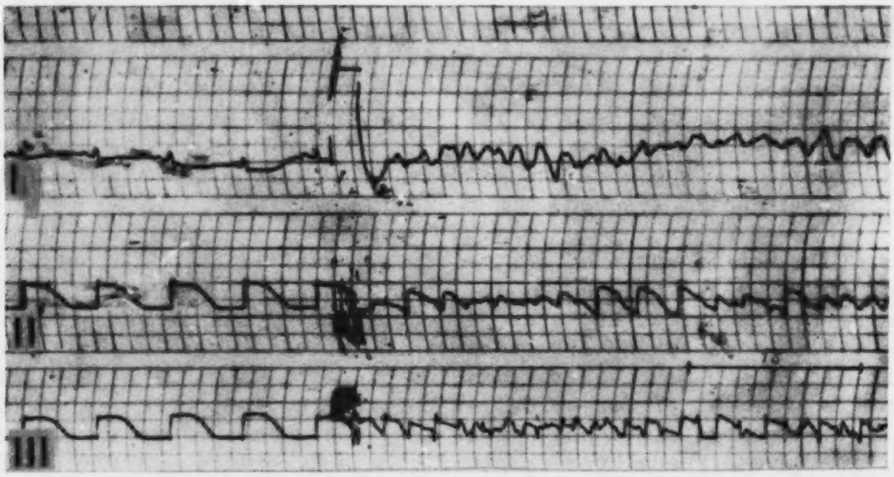

FIGURE 6. Occurrence of irreversible ventricular fibrillation in reaction to a 12 mA pulse train in rabbit 24 hours after reserpine reaction. I = ECG recording. II and III = Electrograms of left and right ventricles, respectively, obtained with sucker electrodes.

myofibrils are indeed connected with this peculiarity of the myocardium of animals treated with reserpine.

At the same time, the gross destructive changes in the myocardium that have been found could also be the end results of the disturbance of the sympathoadrenal mechanisms of the homeostasis of the organism as a whole--something that undoubtedly takes place in the case of acute sympathectomy caused by the injection of reserpine. The gross ultrastructural changes in the myocardium against a background of increased content of glycogen in rabbits at later periods of the reserpine effect are evidently morphological expressions of the considerable disturbances in the metabolic processes in the cells and could be one of the reasons for the reduction in the ventricular fibrillation threshold in the animals. The data we obtained on the increased vulnerability of rabbit heart to fibrillation at later periods during the effect of reserpine agree with the results of other authors. A number of researchers have already established that pretreatment with reserpine increases the tendency to fibrillation in dogs after occlusion of the coronary vessels (15-17). But, it also has been noted that the increased content of glycogen in the myocardium of dogs treated with reserpine did not protect the heart against subsequent ischemic disturbances induced by the distortion of the coronary vessels (17).

Electron-microscopic study also made it possible to reveal that Purkinje cells, as distinguished from myocardium cardiomyocytes, are more resistant to the effect of reserpine. This fact agrees with the findings of other researchers who point to the great resistance of the Purkinje fibers and other parts of the conduction system to various effects (18,19). One can therefore suppose that reduction in the ventricular fibrillation threshold of rabbits at later periods of the reserpine effect is indeed associated with structural changes in the myocardium and not with the conduction system. Moreover, the difference in the resistance to the effect of reserpine between the cardiomyocytes and the Purkinje cells, in turn, could aid in the development of cardiac fibrillation.

CONCLUSION

The results of the study point to two basic stages in the effect of reserpine on rabbit heart. Initially, one observes a change in cell carbohydrate metabolism, in all probability the result of the sharp deficit of catecholamines in the organism caused by the activity of reserpine. At later periods, gross destructive changes in other cellular organelles, particularly in the myofibrils and mitochondria, are added to the disturbance of the carbohydrate metabolism. The gross structural changes in the myocardium could be one of the causes of increased vulnerability of the heart to fibrillation, and could be the reason for the irreversible nature of fibrillation when it occurs. The dissimilarity in the resistance to the effect of reserpine of cardiomyocytes and Purkinje cells may also aid in the development of cardiac fibrillation, and this, in the final analysis, will cause the animals to die.

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PART IV

ANTIARRHYTHMIC DRUGS

PHARMACOKINETICS OF ANTIARRHYTHMIC DRUGS

Raymond L. Woosley and Dan M. Roden

ABSTRACT. The efficacy and toxicity of antiarrhythmic drugs is predictably related to their concentration in plasma. However, the range between these actions is narrow; that is, the toxic concentration usually exceeds the effective level by only twofold or threefold. In addition, the plasma concentration after a given dose may vary widely from patient to patient; factors such as genetics, concomitant drug therapy, or differences in cardiac, renal, or hepatic function may accentuate such differences in a population with arrhythmias. A working knowledge of pharmacokinetics and the determinants of plasma concentration can provide a valuable guide to individualization of initial therapy; thereafter, drug dosage should be adjusted according to clinical outcome and in some cases plasma level monitoring.

Disease states can alter the pharmacokinetics and the response to some agents, such as lidocaine and propranolol in congestive heart failure. Accumulation of active metabolites during chronic therapy (procainamide, propranolol) may confound interpretation of the response to acute drug testing. Clinically important drug interactions such as that recently identified between quinidine and digoxin can best be described and managed using pharmacokinetic principles. Pharmacokinetic evaluation of investigational agents has thus become an integral part of their early clinical evaluation. For example, recent work with encainide HCl has shown the feasibility of dosing every 8-12 hours despite rapid elimination. In addition, varying bioavailability of this agent may have implications for efficacy and side effects. With conventional or

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investigational antiarrhythmic drug therapy, a complete pharmacokinetic data base facilitates rational drug evaluation and patient care.

INTRODUCTION

Symptoms of malignant arrhythmias can often be managed effectively with currently available antiarrhythmic drugs. Even assuming that such agents may prevent sudden death in the large population that is at risk, therapy is inconvenient and frequently limited by toxicity. Because pharmacologic effects (both arrhythmia suppression and toxicity) are related to plasma concentration, therapy should be designed to maintain concentrations above the minimum required for efficacy and below that producing side effects, i.e., within the therapeutic window. This margin between effective and excessive antiarrhythmic drug concentrations is narrow for currently available agents. Knowledge of the pharmacokinetic principles that determine these concentrations in plasma permits individualization of therapy.

After a brief review of the basic principles of pharmacokinetics, specific examples of their importance in antiarrhythmic therapy are given. Finally, we illustrate the value of pharmacokinetic evaluation of investigational agents early during clinical study.

BASIC PRINCIPLES

Once a drug has been absorbed or injected into the body, two basic processes, elimination and distribution, determine its concentration at any given time. Drug elimination is assessed from the measurement of clearance, which is defined as the volume of plasma or blood cleared of drug in unit time. Because clearance has units of flow, it can be related to organ blood flow to give a measure of the efficiency of elimination. For example, if a drug is cleared at a rate of 750 ml/min by the liver, which has a blood flow of 1,500 ml/min, then 50 percent of the drug delivered to the liver is removed in a single pass, so that the extraction ratio is 0.5.

The concentration of a drug (C_{ss}) that has been infused until a steady state is reached depends only on drug clearance (Cl) and the infusion rate (I), as follows:

$$C_{ss} = \frac{I}{Cl}.$$

After administration of multiple, intermittent intravenous doses has led to a steady state, drug clearance and dose (D) also determine the average concentration (C_{av}) achieved and the area under the plasma concentration/time curve (AUC) during the dosage interval so that

$$AUC = \frac{D}{Cl}$$

and

$$C_{av} = \frac{D}{Cl \times \tau}$$

in which τ is the time interval between doses. It can be seen that C_{av} is the same as C_{ss} obtained by constant infusion of D units of drug every τ minutes. Changes in volume of distribution do not affect steady-state drug levels, although this commonly is thought to be the case. Obviously, however, drug concentrations do increase and decrease when drugs are given intermittently, and in this situation volume of distribution can affect the drug concentration present at any particular time and administration.

The volume of distribution of a drug is determined by the relative degree of binding or uptake by the tissues compared with that in blood or plasma where drug is sampled. It is defined as the volume of blood (or plasma) into which the mass of drug in the body (M) must be diluted to result in a given plasma concentration (C), so that

$$V_d = \frac{M}{C}.$$

The concentration of drug at any given time, C_t, is therefore determined both by volume of distribution and by the mass of drug present, which in turn is determined by the dose and the amount eliminated over the time since the drug was first administered. For most drugs elimination proceeds as a first-order process; that is, the rate of elimination is proportional at all times to drug concentration. Under these circumstances drug clearance represents a constant fraction (k) of the volume of distribution (V_d) so that

$$k = \frac{Cl}{V_d}.$$

In fact, k is the first-order rate constant for elimination and has units of $time^{-1}$. This can be converted to the more common expression, drug half-life ($T_{1/2}$), as

$$k = \frac{0.693}{T_{1/2}}$$

so that

$$T_{1/2} = \frac{V_d \times 0.693}{Cl}.$$

This is perhaps the most important of pharmacokinetic equations and assumes that the body is a single homogeneous compartment. It also illustrates the fact that drug half-life depends on both volume of distribution and drug clearance.

To summarize, if a drug is injected rapidly (and we do not generally recommend this) its initial concentration (C_o) is determined by volume of distribution (V_d) and dose (D) according to the equation $C_o = D/V_d$ because little drug has been eliminated in the short time taken to inject it. Thereafter, drug removal is proportional to concentration, which decreases exponentially because the reduction of drug concentration results in a progressive reduction in the amount of drug eliminated in any given time period. Drug half-life is, by definition, the time taken for drug levels to fall to one-half of their initial value, so that after one half-life 50 percent of the drug is left in the body. After another half-life, levels will fall by another 50 percent to 25 percent and 75 percent of the dose will have been eliminated. The amount of drug left in the body after various half-lives is shown in table 1. It can be seen that after five half-lives most of the drug is eliminated.

Drug Accumulation

It takes a finite time after starting a given dose of a drug for plasma concentration to reach its eventual steady-state level. In addition to describing the rate of reduction of drug concentration, the drug half-life also describes the rate of accumulation to a steady state during constant infusion. In fact, the time course of accumulation is the mirror image of that for elimination, for the following reason: Steady state is defined as that situation in which the rate of removal is equal to the rate of delivery of drug so that plasma concentrations remain constant. The percent of the final steady-state levels achieved after various half-lives of infusion is also given in table 1, which shows that more than 90 percent of the steady-state level is reached after three or four half-lives. It should be emphasized that doubling the rate of infusior will not alter the time taken to obtain the steady state but will double the drug concentrations achieved.

TABLE 1. Effects of Half-Life on Drug Elimination and Accumulation

Half-Lives (No.)	% Remaining*	% Accumulation**
1	50	50
2	25	75
3	12.5	87.5
4	6.25	93.75
5	3.125	96.875

*The fraction of the amount in the body or initial plasma level present various times after a single dose.

**The fraction of the final steady-state drug concentration achieved at various times during continuous drug administration.

Loading and Maintenance

Single intravenous loading dose. Because the time taken to reach a steady state may be too long in patients requiring urgent treatment, use of loading and maintenance doses is often required, as in the case of the cardiac glycosides. There are several ways to achieve the desired effect relatively rapidly. The first involves use of a single loading dose. In the case of antiarrhythmic drugs given intravenously, this method is not satisfactory because the peak levels attained are usually associated with adverse effects. This situation occurs because the concentration/time profile of these drugs after intravenous administration is, in fact, biexponential--an early rapid phase of reduction being followed by a slower decrease in concentration. In this situation the body acts as if it were composed of two compartments, a central one that reflects plasma concentration and from which elimination occurs, and a peripheral one that comprises those tissues that equilibrate slowly with plasma (figure 1). As a result, immediately after injection, drug concentration (C_0) is determined by the dose (D) and the

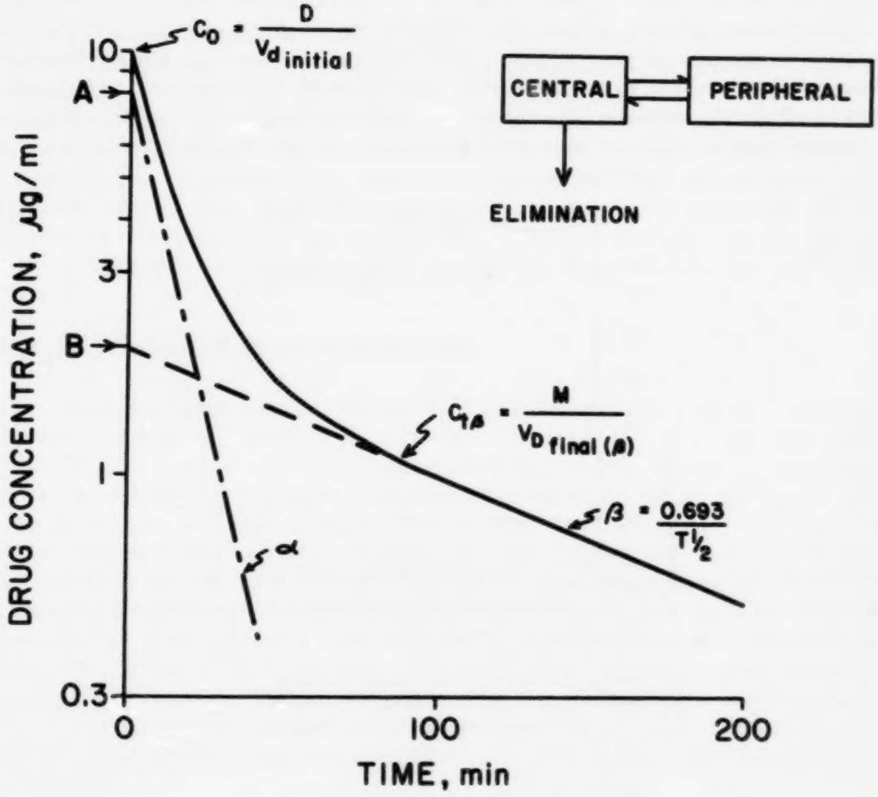

FIGURE 1. Drug concentrations after intravenous administration show a biphasic curve (that is, two-compartment system). $C_{\tau\beta}$ = Final drug concentration. C_0 = Initial drug concentration. D = Dose. M = Mass of drug in the body. $T_{½}$ = Half-life. $V_{d\ initial}$ = Initial volume of distribution. $V_{d\ final(\beta)}$ = Final volume of distribution. (Reprinted with permission from Woosley RL and Shand DG: Pharmacokinetics of antiarrhythmic drugs. Am J Cardiol 41:986, 1978)

volume of the central compartment or the initial volume of distribution (V_d) ($C_o = D/V_d$). Because the drug distributes out of this compartment into the tissues at the same time as it is eliminated, the measured volume of distribution increases from its initial value and drug concentrations fall rapidly to reach a value determined by the mass of the drug in the body and the volume of distribution at the time the late phase of elimination becomes a single exponential. This is the final volume of distribution ($V_{d\beta}$).

Loading dose followed by maintenance infusion. If we try to give such a drug as a single dose followed by an infusion designed to give steady-state levels in the middle of the therapeutic range, two equally undesirable effects may be seen (figure 2). When the single dose is adjusted to avoid peak levels in the toxic range, plasma levels "dip" below therapeutic levels for a time before the concentrations due to the infusion rise into the therapeutic range. This situation occurs because the rate of rise of concentrations due to the infusion is determined largely by the terminal or β-phase half-life. In the example used (figure 2, top panel) the half-life is 100 minutes so that levels due to the infusion would have risen to only 30 percent of steady state by 50 minutes, at which time levels after the injection would have fallen to 30 percent of their initial value of 10 μg/ml. The resultant sum of these two therefore falls below minimal effective levels. The second alternative of giving a large enough single dose to ensure therapeutic levels throughout is just as unsatisfactory because toxic levels may be attained for an appreciable time (figure 2,

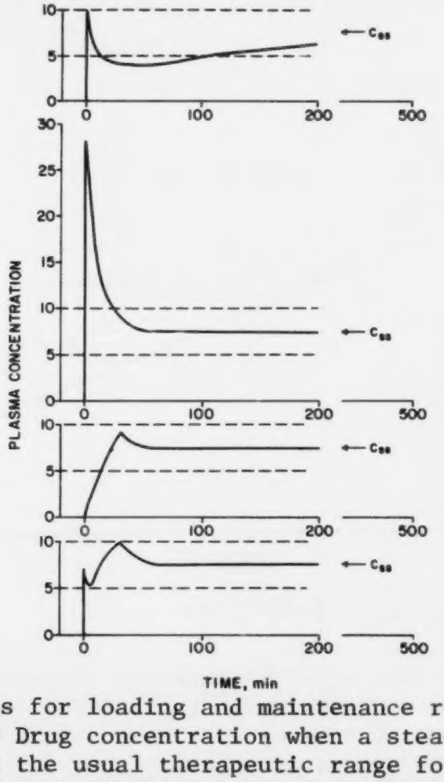

FIGURE 2. Alternatives for loading and maintenance regimens after intravenous administration. C_{ss} = Drug concentration when a steady state is achieved. The dotted lines represent the usual therapeutic range for a hypothetical antiarrhythmic drug. (Reprinted with permission from Woosley RL and Shand DG: Pharmacokinetics of antiarrhythmic drugs. Am J Cardiol 41:986, 1978)

second panel). Put another way, the difference between peak and equilibrium levels (which is dependent on the ratio of initial and final volumes of distribution) exceeds the therapeutic ratio.

Double infusion technique. To avoid these problems, a double infusion technique, whereby a rapid infusion is followed by the maintenance infusion, has been advocated (1). Although this type of regimen is available for all of the antiarrhythmic drugs, safety is achieved at the expense of the time taken to reach the therapeutic range (figure 2, third panel). While the double infusion technique is often satisfactory, urgent treatment can be initiated and maintained by giving a single injection followed by the double infusion (figure 2, bottom panel). However, it should be stressed that the single dose or rapid infusion rate, or both, should be reduced because plasma concentrations will be the sum of the two.

Oral Administration

After oral administration the distribution phase is generally obscured by the absorptive phase and can be ignored for practical purposes. The factors influencing drug elimination and volume of distribution are the same as those previously described, but in addition we must consider the fraction of the dose that reaches the systemic circulation and then the target organ--that is, systemic availability. Availability may be reduced by (a) incomplete absorption, and (b) metabolism by the intestine or liver, during the passage of drug from the gut lumen to the systemic circulation. The latter effect is called presystemic or "first pass" elimination and occurs with several antiarrhythmic drugs.

APPLICATION OF PHARMACOKINETIC PRINCIPLES

Widely quoted therapeutic ranges for antiarrhythmic drug concentrations are only rough indicators of desirable levels, especially for any individual patient (figure 3). Their limits are the concentration above which a desirable effect is likely to be observed and the concentration below which adverse reactions are unlikely to occur in a large proportion of the population. Clearly the definition of this therapeutic window depends on the incidence and severity of adverse reactions, as well as on the endpoint used to designate antiarrhythmic response. A narrow range is observed if total arrhythmia abolition is the therapeutic goal, and absolutely no side effects are allowed; if abolition of complex arrhythmias is satisfactory, even with a modicum of mild side effects, the therapeutic range is correspondingly wider. Such ranges are only guidelines and should be used in conjunction with clinical judgement: Responses in a single patient may vary from those predicted by guidelines established for the entire population of patients with arrhythmias.

Because the range between effective and excessive antiarrhythmic plasma concentrations can be quite narrow and because the elimination of these agents can be rapid, dosing must be carried out frequently. For instance, the therapeutic range for procainamide is 4-8 μg/ml. Since its elimination is so rapid, dosing must be carried out every 3-4 hours (2). If the drug is administered less often, concentrations that are subtherapeutic will occur prior to a dose, as well

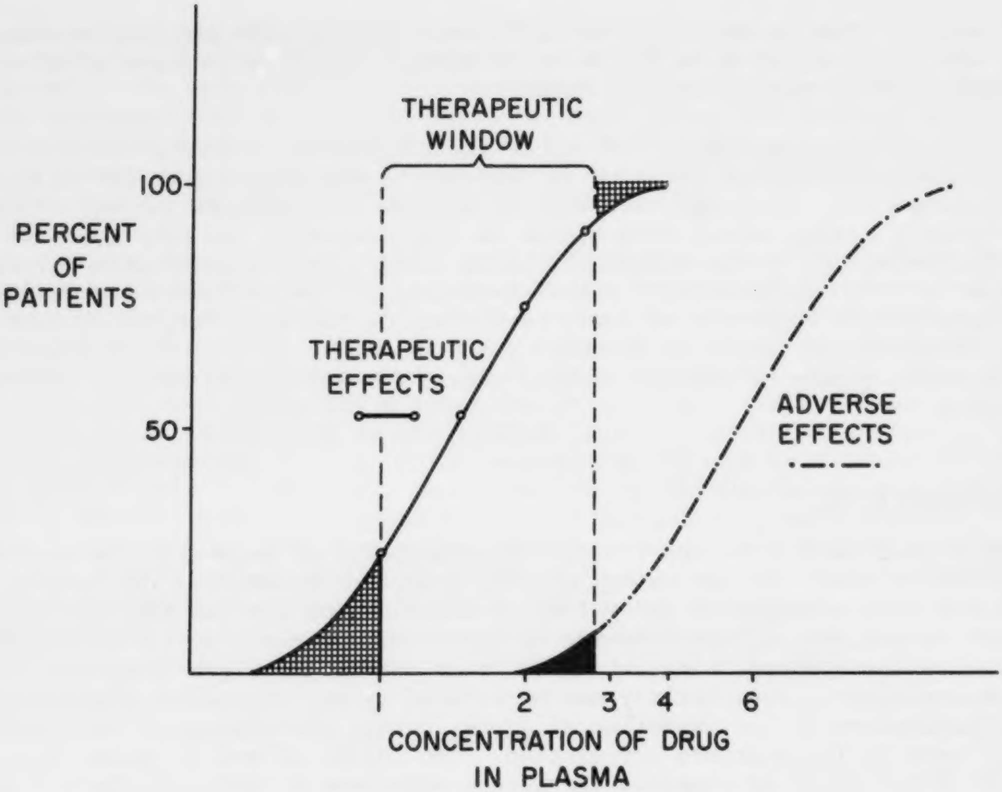

FIGURE 3. Dose-response curves for therapeutic effects and side effects of a drug graphed to demonstrate the therapeutic window. (Reprinted from Oates JA and Wilkinson GR: Chapter 66, The principles of drug therapy. In Principles of Internal Medicine, edited by Harrison, 1977, p 345. Used with permission of McGraw-Hill Book Company, New York)

as potentially toxic concentrations following a dose (figure 4). While this holds true for most patients, dosing every 6 hours is occasionally feasible but only after careful evaluation reveals no increased arrhythmia at the end of the dosing interval and no unacceptable side effects. It also follows that patients whose elimination of this agent is impaired may well tolerate dosing every 6 hours. This occurs in renal failure where small doses of procainamide given as infrequently as every 12 hours may produce plasma concentrations within the therapeutic range for the entire dosing interval (3).

The therapeutic range for lidocaine plasma concentrations is also only a guide to therapy. While the usual range associated with efficacy is 1.5-5 μg/ml, a subset of patients will respond only at higher (5-9 μg/ml) concentrations (4). The incidence of side effects will be higher in this range, but an individual patient may occasionally require these concentrations and exhibit only mild, if any, side effects.

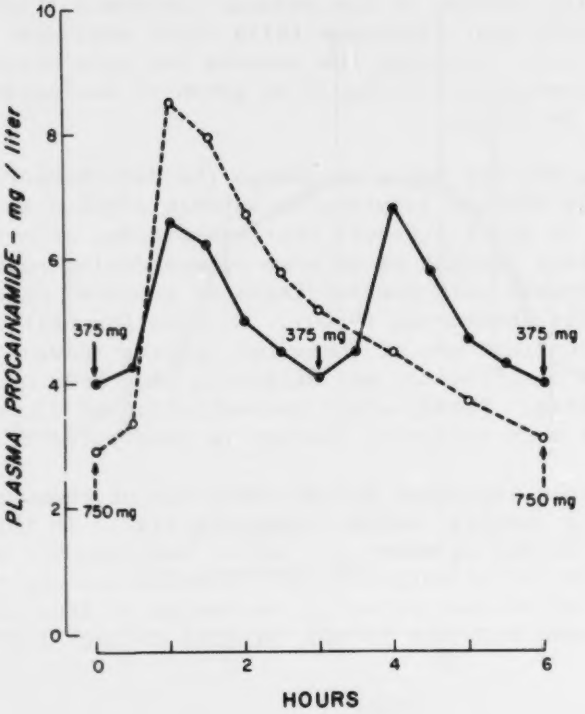

FIGURE 4. Comparison of plasma concentrations obtained after two different regimens of the same total dosage (375 mg every 3 hours vs. 750 mg every 6 hours). The usual therapeutic range is 4-8 μg/ml. [Reprinted with permission from JAMA 215:1454-1460, 1971 (reference 2). Copyright 1971, American Medical Association]

Factors Influencing Drug Disposition

Several factors are known to alter the clearance and distribution of antiarrhythmic compounds in both health and disease. The biological determinants of drug disposition are (a) the activity of the basic removal process (intrinsic clearance), (b) organ blood flow, and (c) drug binding in the blood and tissues. All of these activities may be influenced by constitutional and environmental factors in apparently healthy people, as well as by heart failure, renal failure, and liver disease. Consequently, it is the rule that plasma concentrations vary widely (up to tenfold to twentyfold) when the same dose is given to a group of people. However, the effects of all of these factors can be judged on the basis of the known pathophysiology of disease and the mechanism responsible for the disposition of any given drug. With this information, dosage regimens can be simply designed to fit the needs of any given patient, at least as a first approximation. For instance, lidocaine is so effectively metabolized in the liver that its clearance is essentially limited by its delivery rate (liver blood flow) (5). Disease states can alter the volume of distribution (the determinant of the size of the loading dose) or liver blood flow (clearance). Congestive heart failure is accompanied by a decrease in both these parameters (6); half-life may be only moderately increased, but the use of conventional doses may produce marked toxicity because of the reduced clearance (figure 5). It has been recognized recently that clearance falls after prolonged (> 24 hours) lidocaine administration (7). Although the reasons for this phenomenon are not completely understood, consideration should be given to decreasing maintenance infusion rates after 24 hours.

Concomitant drug therapy may also change the determinants of plasma concentration. The recently defined interaction between digoxin and quinidine provides a striking example. It is of interest that ventricular arrhythmias attributed to quinidine have long been thought to be more common during coadministration of digoxin (8), but that recent work has incriminated elevated digoxin (not quinidine) concentrations for this phenomenon (9,10). Elimination half-life of digoxin is unchanged during concomitant use of these two agents. However, marked reductions in both the volume of distribution and clearance have been observed; the exact mechanism is speculative. Steady-state concentration of digoxin (a function of clearance) thus rises when quinidine therapy is instituted (figure 6).

Quinidine clearance increases during phenytoin or phenobarbital therapy, probably as a result of hepatic enzyme induction (11). In this setting larger than usual doses (up to 800 mg every 4-6 hours) must be used to generate quinidine concentrations in the effective range. Concomitant use of lidocaine and propranolol illustrates another potential mechanism of drug interaction. Although this has not been examined in man, studies in dogs indicate that the

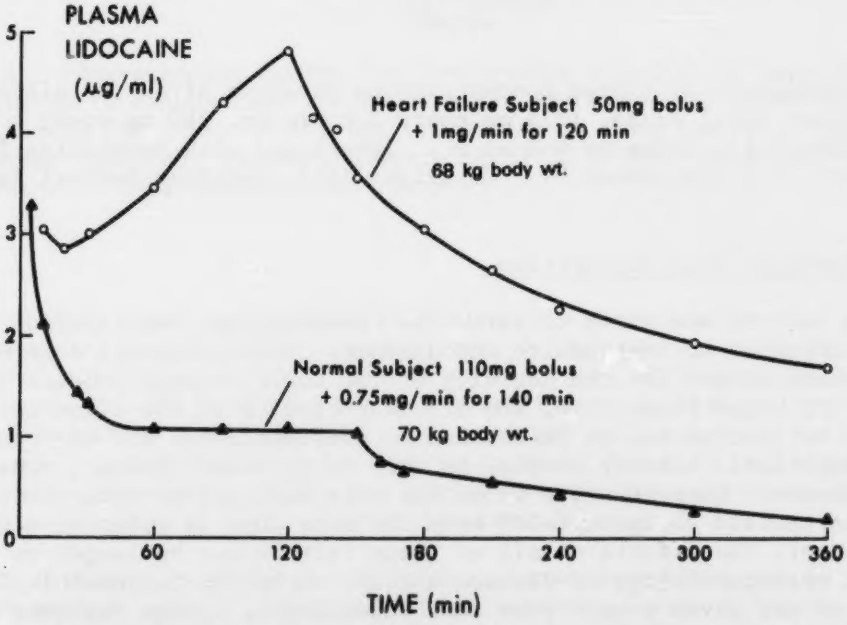

FIGURE 5. Comparison of plasma lidocaine concentrations in a normal subject and in a patient with congestive heart failure. (Reprinted by permission of the American Heart Association, Inc., from Collingsworth KA, et al.: The clinical pharmacology of lidocaine as an antiarrhythmic drug. Circulation 50:1217, 1974)

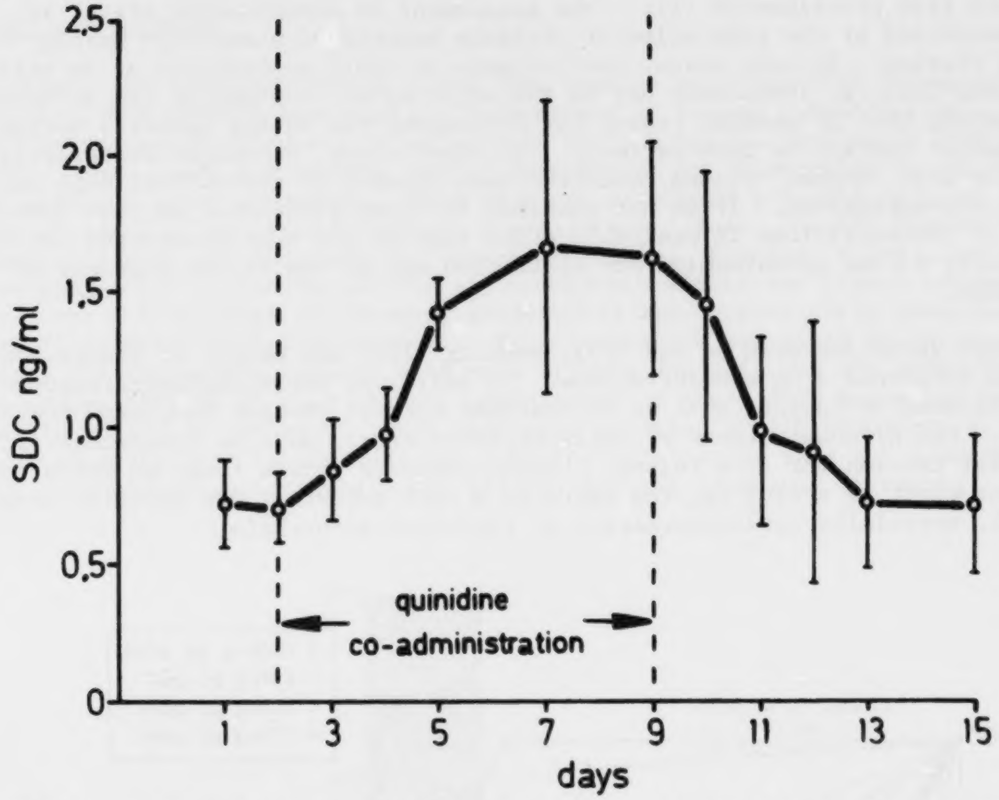

FIGURE 6. Influence of co-administration of quinidine and digoxin on serum digoxin concentration (SDC). [Reprinted by permission from The New England Journal of Medicine 300:400-404, 1979 (reference 9)]

hemodynamic changes secondary to propranolol therapy (reduced cardiac output and presumably reduced hepatic blood flow) correlated well with reduction in lidocaine clearance (12).

Polymorphic routes of metabolism may play a role in determining toxicity or antiarrhythmic effect. For instance, procainamide is metabolized by deacetylation to N-acetylprocainamide (acecainide), which has antiarrhythmic activity of its own (13). The rate of this conversion is genetically determined; about one half of the white and black populations in the United States are "fast" acetylators and one half are "slow," while 70-90 percent of the Oriental populations are "fast" acetylators (14). It has been observed that slow acetylators are more likely to develop antinuclear antibodies, and thus may be at increased risk for the syndrome of drug-induced lupus (15). As might be expected, however, administration of acecainide (which is not significantly deacetylated to procainamide) has not produced antinuclear antibodies or lupus (16,17).

Acecainide's electrophysiological properties (18), its range of therapeutic concentration (9-19 µg/ml), and its elimination half-life (7-10 hours) are different from procainamide (17). The assessment of procainamide effect is thus confounded by the generation of variable amounts of acecainide during chronic therapy. In some cases, particularly in rapid acetylators or in patients with renal failure, acecainide may be the major agent responsible for arrhythmia suppression; this is another reason for prolonging the dosing interval during procainamide therapy in such patients. In other cases, procainamide toxicity may occur with "normal" plasma concentrations because of concomitant high acecainide concentrations. It is not possible to offer guidelines on what combination of concentrations is desirable other than to say that unexpected efficacy or toxicity during procainamide administration may be due to the presence of acecainide.

Other variables such as age (19), smoking (19), and extent of absorption (20) can influence drug concentrations. In addition, antiarrhythmic response can be altered by factors such as hypokalemia without changes in plasma concentration. Use of combinations of antiarrhythmic agents must be approached with particular caution for this reason. Little evidence exists today on which combinations might be effective, and there is a real potential for additive toxicity (21), especially cardiodepression or increased arrhythmia.

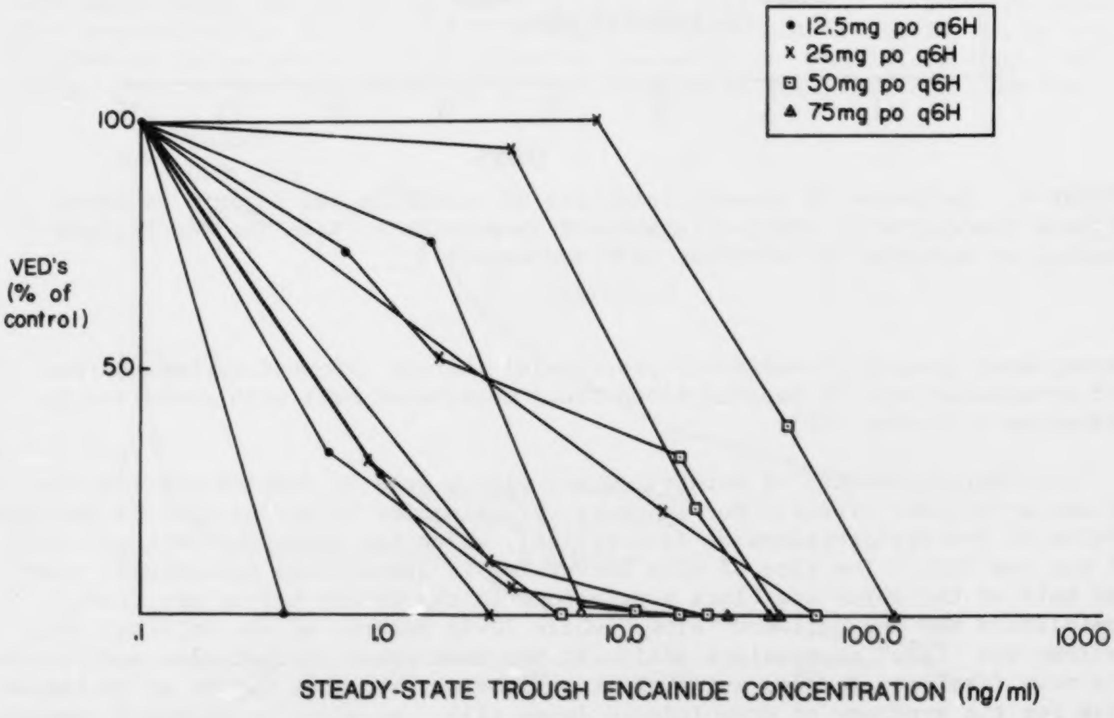

FIGURE 7. Relationship between steady-state trough encainide concentration, arrhythmia frequency, and increasing dosage in 10 patients with ventricular arrhythmias. VED's = Ventricular ectopic depolarizations.

FUTURE APPLICATIONS

Since pharmacokinetic principles have come to be recognized as important guidelines for optimal drug therapy, assessment of plasma concentrations and their determinants becomes a major part of early clinical studies in drug development. Such study can provide useful information as illustrated by our recent work with the agent encainide. This drug was developed a decade ago, but because of rapid elimination following a single large dose, chronic therapy on a practical dosing schedule was not thought feasible. However, using a protocol which called for evaluation of the antiarrhythmic effect and plasma concentrations over a wide range of dosages, arrhythmias were reproducibly abolished in 12 of 13 patients tested (figure 7). The elimination was rapid in these patients, but the margin between mild side effects and efficacy was sufficiently wide enough (figure 8) that chronic administration every 6-12 hours has been possible in all 12 patients. This contrasts strikingly with procainamide and illustrates the need to consider not only the elimination rate but also the width of the therapeutic range when determining the frequency of dosing.

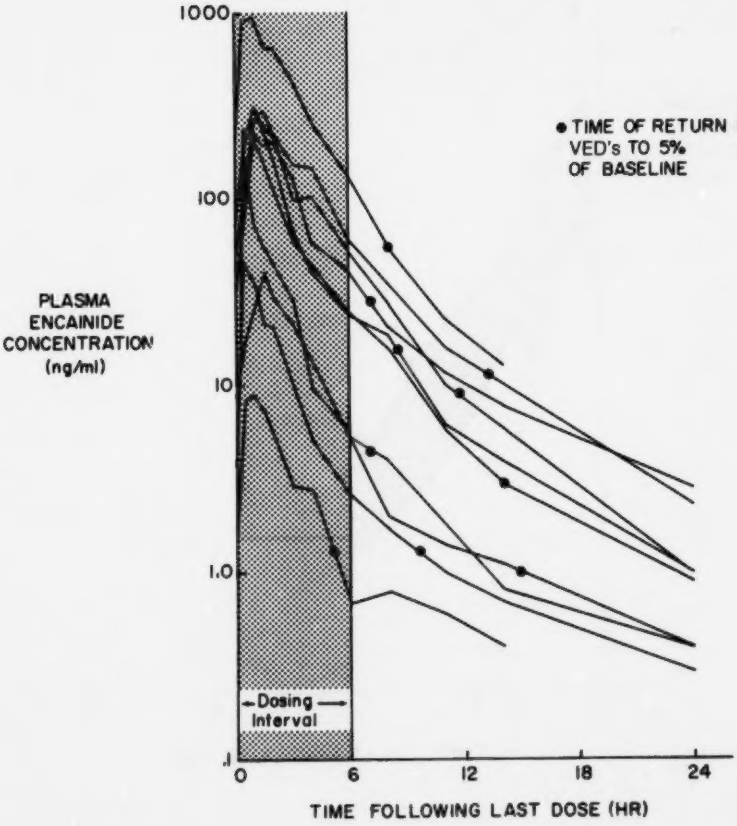

FIGURE 8. Recurrence of ventricular arrhythmia after discontinuation of 6-hourly encainide therapy (n = 10). VED's = Ventricular ectopic depolarizations.

Another important observation during this trial was that the pattern of elimination of encainide was aberrant in one patient. Her elimination rate was much slower (figure 9) and her steady-state concentrations were much higher than other patients. Such patients may be at increased risk of unexpected drug action.

CONCLUSION

Empirical administration of conventional dosages of antiarrhythmic agents does not take into consideration individual variations in pharmacokinetics or

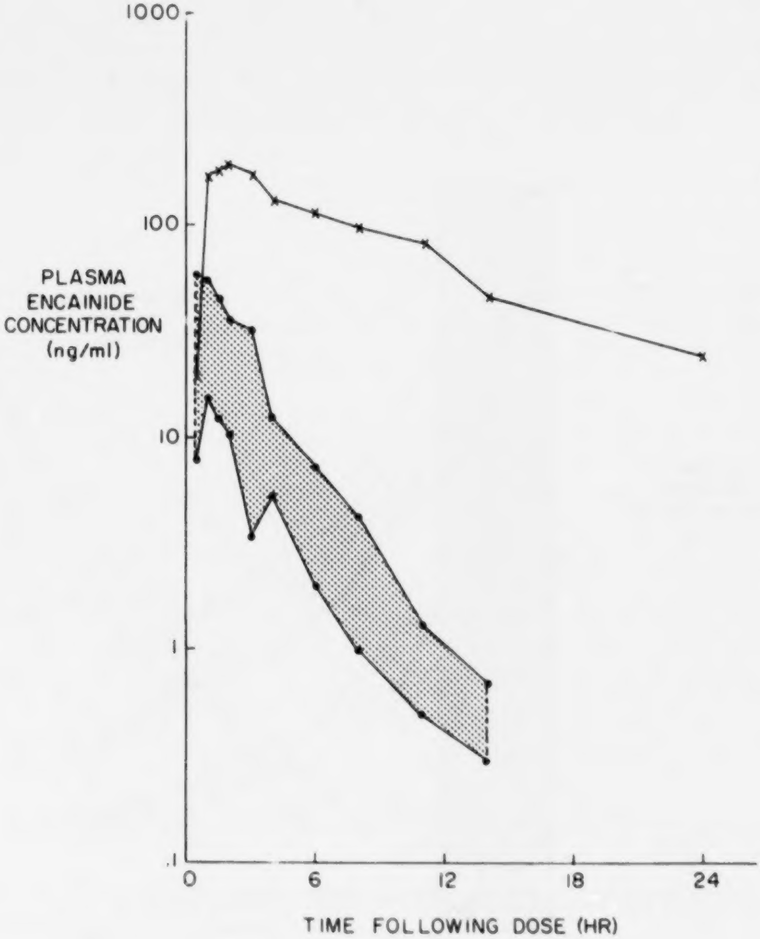

FIGURE 9. Abberrant elimination pattern in one patient following a single oral 25 mg dose of encainide. The shaded area represents the 95 percent confidence limits of the mean for 10 other patients.

complicating factors which may alter them. Knowledge of the pharmacokinetic determinants of drug effects and the conditions under which these determinants may deviate from the usual is a better guide to drug therapy. Monitoring plasma concentration should be an adjunct to therapy, particularly when unexpected toxicity or drug resistance occurs. Future antiarrhythmic drug therapy, whether with currently available agents or with newly developed ones, requires a complete pharmacokinetic data base.

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SYMPATHETIC CONTROL OF HEART AND VESSELS: ANTIARRHYTHMIC AND ANTIANGINAL DRUG EFFECTS

N. V. Kaverina, N. V. Darinsky, and I. N. Pidevich

ABSTRACT. The influence of antianginal (nitroglycerin, nonachlazine) and antiarrhythmic (ethmozin, lidocaine, mexiletine) drugs on the tonic activity and reflex discharges in the sympathetic cardiac nerves in response to stimulation of the afferent fibers of the somatic and visceral nerves was investigated. The effects of these drugs on the reflexes from the heart under chemical stimulus (coronary chemoreflex) were also studied. The 10-aminoacyl derivatives of phenothiazine (nonachlazine, ethmozin and its diethylamine analog) are shown to have a selective influence on the reflex response from high-threshold A fibers conducting "primary" pain signals to the central nervous system. Nonachlazine acts like nitroglycerin. Lidocaine and mexiletine have a marked inhibitory effect on tonic and reflex sympathetic activity. Responses to an impulse transmitted by the afferent C fibers are somewhat more sensitive to the action of these drugs (with effects similar to those of morphine). Of the drugs studied, only nonachlazine is capable of blocking the coronary chemoreflex to serotonin. It is suggested that the effect on sympathetic control of the heart and vessels may be significant in the realization of the cardiovascular effects of the drugs studied.

INTRODUCTION

Emotional stress leading to the activation of sympathetic influences on the heart and coronary vessels is one of the important factors predisposing to the development of cardiac fibrillations. The importance of intensified activity of the sympathetic nervous system to the development of arrhythmia in acute myocardial ischemia has been studied by many researchers using various methodological approaches. Brown (1), Brown and Malliani (2), Malliani et al.

From the Institute of Pharmacology, USSR Academy of Medical Sciences, Moscow, USSR.

(3), Gillis (4), and Uchida and Murao (5) have established that myocardial ischemia leads to the excitation of the cardiac sympathetic afferent fibers, and to the genesis of cardiac reflexes. A number of researchers have reported that stimulation of the stellate ganglia in conditions of myocardial ischemia significantly reduces the fibrillation threshold (6-8). Removal of the stellate ganglia or injection of β-adrenoblockers prevents reduction in the fibrillation threshold caused by excitation of the sympathetic nervous system.

Stimulation of the vagus nerve, on the other hand, raises the threshold of ventricular fibrillation. This effect, however, can to a great extent develop with an increase in the activity of the sympathetic nervous system (8-11). Intensification of the vagus effects on animal hearts treated with β-adrenoblockers does not, for all practical purposes, change its vulnerability to fibrillation. Thus, the positive effect of the vagus nerve can be regarded as indirect and caused by the relative reduction of sympathetic influences on the myocardium.

These data provide the basis for proposing that the use of drugs that influence the various links of the reflexes responsible for regulation of circulation and cardiac activity can, in different ways, play an important role in the development of methodological approaches to the prevention and treatment of life-threatening disturbances in cardiac rhythm. The effect of the drugs used to treat ischemic heart disease (IHD) on nerve control of the heart and vessels has not been studied systematically, however.

The objective of the present work was, therefore, to study the effect of a number of antianginal and antiarrhythmic drugs on the tonic and reflex activity in the sympathetic cardiac nerves upon electrical stimulation of the afferent fibers of the somatic and visceral nerves. The effect of the drugs on coronary chemoreflex, manifested by an increase in the tone of the vagus nerve and by a reduction in sympathetic activity, bradycardia, and hypotension, was investigated in individual series of experiments. This reflex originates with the cardiac receptors when influenced by such agents as veratrum alkaloids, derivatives of guanidine and thiourea, sodium salicylate, and, particularly important, serotonin (12,13). Precipitating from the thrombus into the vessel lumen in myocardial infarction, serotonin can cause coronary chemoreflex. The latter, leading to bradycardia and inhibition of sympathetic activity, can reduce the vulnerability of the heart to fibrillation.

MATERIALS AND METHODS

Cats were used for the experiments. The animals were anesthetized with urethane (400 mg/kg) and chloralose (30 mg/kg) under artificial respiration. Arterial pressure was recorded from the femoral artery by electric manometer.

The functional state of the sympathetic nervous system was determined during the first part of the study by changes in the spontaneous bioelectrical activity and reflex responses in the inferior cardiac, or renal, nerves. The afferent neurogram was used to determine the involvement of various groups of fibers (14). The instrument used for the recording was the Mingograf-81. In a series of experiments, amplified bioelectrical discharges were fed through a bridge rectifier to an ATAC-350 analyzer which produced an average of 20 responses at a frequency of 0.3-0.5 pps. Stimulation of the central segments of the somatic and visceral nerves by electrical stimuli at 1-3 V, for a duration of 0.1-0.2 msec, caused a cardiac nerve reflex response from the afferent A fibers with a latent period of 60-110 msec and a duration of 50-130 msec. This response has a long (300-500 msec) inhibitory component manifested by inhibition of the tonicity of the active preganglionic neurons. An increase in the force of the stimulation to 10-20 V and to a duration of 1 msec caused the appearance in the sympathetic nerves of yet another component, that of a reflex response to an impulse from the afferent C fibers with a latent period of 300-500 msec and a duration of 200-600 msec.

The pain syndrome is, as known, one of the main signs of angina pectoris, as well as of significant pathogenetic value in IHD. The functional roles of the reflex discharged from the A and C afferent fibers differ. The high-threshold A fibers are responsible for conducting the so-called "primary" pain pulses, whereas the C fibers transmit the signals of the almost undifferentiated "secondary" pain. Study of the influence of drugs on each of the components of the reflex responses in the cardiac sympathetic nerves was therefore of interest.

The second part of the work investigated the effect of drugs on coronary chemoreflex as caused by serotonin, veratrine (a mixture of veratrum alkaloids), α-naphthyl biguanide, or sodium salicylate. Agents causing coronary chemoreflex were injected intravenously or into the cavity of the cat's left ventricle through a catheter led through the subclavian artery and the ascending aorta (13). We investigated the effects of drugs already known and in clinical use, such as nitroglycerin, lidocaine, and mexiletine, as well as new, original, agents synthesized and studied at the Institute of Pharmacology of the USSR Academy of Medical Sciences. The latter agents were 10-aminopropionyl derivatives of phenothiazine: an antiarrhythmic agent (ethmozin), its diethylamine analog (ethmozin DAA), and an antianginal agent (nonachlazine).

RESULTS AND DISCUSSION

Nitroglycerin is one of the oldest drugs used to treat angina pectoris, yet even today it remains the most effective antianginal agent and, in fact, its use is increasing. Nitroglycerin has, in recent years, begun to be used successfully intravenously to treat acute myocardial infarction. It is a known fact that nitroglycerin can improve the function of the left ventricle and reduce ventricular arrhythmia during acute myocardial infarction (15, and others). Experiments in dogs with acute coronary occlusion showed that nitroglycerin increases the electrical stability of the heart (16,17) and reduces the incidence of spontaneous ventricular fibrillation (18).

Our experiments revealed that nitroglycerin administered in a dose of 200-500 μg/kg intravenously inhibited the tonic activity and reflex responses in the cardiac sympathetic nerves. At the same time, the drug exhibited a selective inhibitory effect on the intensity of discharges initiated by impulses from the slowly conducting high-threshold fibers of the A group (A - δ and post-δ), intensifying the inhibition of the tonic activity after these discharges. The reflex responses to the impulse from the C afferents were practically unchanged, however (figure 1). As shown in this figure, morphine, used for comparative purposes as a typical analgesic, differs from nitroglycerin in that it has a selective effect on reflex C responses.

The effect of nitroglycerin on tonic and reflex activity in the sympathetic nerves, as well as on vasomotor reflexes when the labile store of monoamines has been exhausted by reserpine, was investigated in order to analyze the central neurochemical mechanisms responsible for the display of the effects described. Nitroglycerin had no effect after reserpine administration, so the effect is associated with the presence of free monoamines in the cerebral tissue. Actually, free norepinephrine appeared in the cerebrospinal fluid during

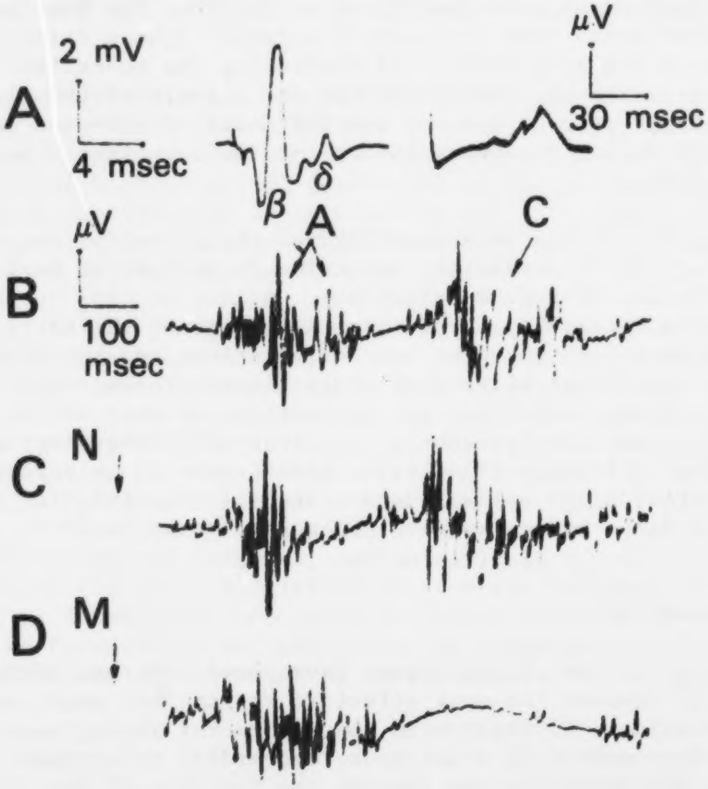

FIGURE 1. Influence of nitroglycerin and morphine on the reflex responses in the inferior cardiac nerve and on stimulation of the tibial nerve. A: Left = Afferent neurogram of fibers of the tibial nerve during stimulation of the afferent fibers of the A and C groups (stimulation parameters 1 V, 0.2 msec). Right = Same during stimulation of the afferent fibers of the C group (15 V, 1 msec). B: Reflex responses during stimulation of the afferent fibers of the A and C groups (15 V, 1 msec). C = Same 15 minutes after intravenous injection of nitroglycerin (N) (0.1 mg/kg). D = Same 8 minutes after intravenous injection of morphine (M) (0.3 mg/kg).

the period of inhibition of the vasomotor reflexes, but its content in brain and heart tissue was reduced after 30 minutes (19).

Analysis of the central effect of nitroglycerin established the significant intensification of its inhibitory effect on the sympathetic tonus in experiments on decerebrated cats (dissected at the boundary of the pons and medulla oblongata), and, conversely, the absence of this effect in experiments on spinal animals. These data, as well as the similarity between the effects described and the effect of norepinephrine (when injected into the lateral ventricle of the brain), or DOPA intravenously (20), permit the hypothesis that nitroglycerin, by releasing catecholamines, causes activation of the processes of suprasegmental reticulospinal inhibition. The selectivity of its effect on reflex responses initiated by impulses transmitting signals of "primary" (possibly visceral) pain is evidently the result of activation of individual monoaminergic descending pathways oriented in a segmental system of Aδ afferents (21).

Experiments designed to study the effect of nitroglycerin on the coronary chemoreflex initiated by various stimuli established that this reflex is not changed by the influence of nitroglycerin administered intravenously at a dose of 100-500 μg/kg.

Thus, the effect of nitroglycerin on cardiac nerve control is expressed in its inhibitory effect on the central processes of regulation of the sympathetic tone. These effects are associated with the interference of nitroglycerin in the metabolism of catecholamines and is evidently accomplished by free epinephrine. In light of these ideas, it is of interest to review the data presented by a number of authors who have pointed out that nitroglycerin raises the threshold of electrical fibrillation in dogs with ischemic myocardium (16) and with nonischemic myocardium (22) by a factor of almost 2. The authors leave open the question of what the mechanism is that is associated with the rise in the cardiac fibrillation threshold attributed to nitroglycerin. It should be noted that the increase in cardiac electrical stability attributed to nitroglycerin is seen under conditions of coronary occlusion (17), that is, at a time when intensification of sympathetic effects on the myocardium are particularly important to the development of fibrillations (8). One can therefore assume that the central inhibition of sympathetic effects on the heart can play an important role in the mechanism by which nitroglycerin increases cardiac electrical stability.

Nonachlazine--10-β[1,4-diazo bicyclo-(4,3,0)-nonanilpropionyl-4]-2-chlorphenothiazine dichlorohydrate--is a new antianginal preparation synthesized and studied at the Institute of Pharmacology of the USSR Academy of Medical Sciences (23-26). Nonachlazine is one of a new class of antianginal drugs, whose special feature is the presence of a β-adrenostimulating component in the spectrum of its effects. The main antianginal effect of the drug is its ability to redistribute the blood flow to favor the myocardial ischemic zone and to improve its functional state (25). Nonachlazine, thanks to the presence of the β-adrenostimulating effect, improves myocardial contractility. However, as distinguished from "pure" β-adrenostimulators, the drug does not induce tachycardia, nor does it increase oxygen absorption by the heart. These properties evidently depend on the ability inherent in nonachlazine to reduce the reaction of the heart to isoproterenol, along with β-adrenostimulation, and thus cause partial blockage of the β-adrenostructures. Nonachlazine, moreover, had a negative inotropic and chronotropic effect in experiments with an isolated atrial appendage. This effect remains unchanged when the adrenergic and cholinergic structures are blocked, and so depends on the direct inhibitory effect of nonachlazine on the myocardial cell (27). These effects of nonachlazine determine to a large degree the drug's adrenergic properties. Nonachlazine blocks mediator uptake and competes with the latter for a bonding site on the membrane (28). It has been hypothesized that the partial β-adrenergic blockage caused by nonachlazine is what helps it to occupy a site on the membrane and that this is because of its greater affinity for the membrane.

Nonachlazine has antiarrhythmic properties. It approaches ethmozin in terms of its spectrum of antiarrhythmic effects, but is much less effective (by a factor of 5-6) than ethmozin activity on the majority of arrhythmia models (23).

The influence of nonachlazine on tonic activity and reflex responses in the cardiac sympathetic nerves depends on the doses used. A 1 mg/kg dose facilitates reflex responses to impulses from the afferent A and C fibers, diminishing the inhibition of tonic activity after discharges. On the other hand, a 6 mg/kg dose, which demonstrates the drug's principal antianginal and antiarrhythmic properties, inhibits spontaneous activity and reflex responses in the sympathetic nerves, as well as pressor reflexes. Its inhibitory effect is largely seen with respect to the long-latent, high-threshold part of the discharges from the A afferent fibers (figure 2). The nature of the effect of nonachlazine at this dosage is close to that of nitroglycerin.

The purpose of the experiments was to analyze the role of the central adrenergic processes in the realization of the effects of nonachlazine. In some of the experiments, reserpine was used prior to testing, and in others propranolol was injected (at a dose of 5 mg/kg intravenously). We found that small doses of nonachlazine showed no facilitating effect on the depletion of labile stores of cerebral catecholamines, but that the inhibition characteristic of large doses continued. The β-adrenergic blockage prevents development of the activating, as well as the inhibiting, effect of nonachlazine. It has already been demonstrated that norepinephrine (when injected into the ventricle of the brain), or its precursor DOPA (intravenously), had an activating effect on the sympathetic tone when administered in small doses, and an inhibitory effect when administered in large doses. The latter is associated with activation of the processes of suprasegmental inhibition (20).

One can therefore suppose that the activating effect of nonachlazine on the sympathetic tone is through the catecholamines, whereas the inhibitory effect depends on the drug's direct stimulating influence on the β-adrenostructures of the monoaminergic inhibiting neurons. In the case of decerebrated animals, nonachlazine in small doses showed an unrewarding influence on the excited and inhibited components of the reflex A response. An increase in the dosage to 6 mg/kg completely suppressed discharges from the high-threshold A fibers and made initiation of C discharges difficult. A 6 mg/kg dose of nonachlazine initiated inhibitory effects of lesser intensity in experiments with spinal animals than was the case with decerebrated animals.

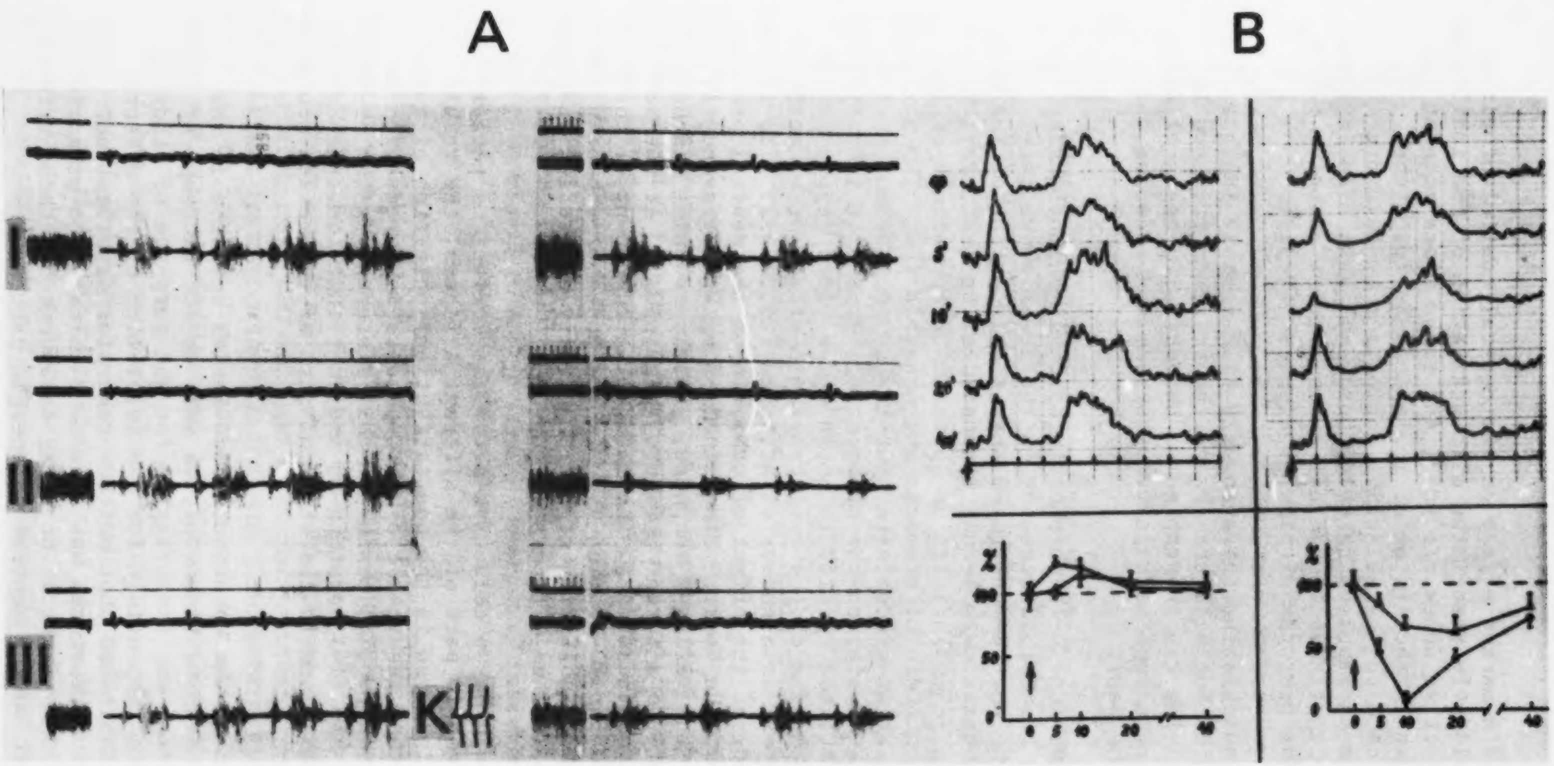

FIGURE 2. Influence of nonachlazine on tonic activity and reflex responses in renal nerve upon stimulation of the tibial nerve. A: Recording on a Mingograf-81 (Elema, Sweden) of tonic activity and reflex responses upon rhythmic stimulation of the tibial nerve A and C afferent fibers (15 V, 1 msec). I, II, III, top to bottom: Time ticks 1 sec, stimulation, electrogram in renal nerve. Left = After 1 mg/kg intravenously. Right = 6 mg/kg. I = Control. II = 10 minutes. III = 40 minutes after injection. K = Calibration 100 μV. B: Recording on ATAC-350 (Nikkon-Koden, Japan). Averaged (20 stimulations, interval 4 sec) identical responses in renal nerve upon stimulation of the tibial nerve A and C afferent fibers (15 V, 1 msec). Left, top to bottom: background 5, 10, 20, and 40 minutes after 1 mg/kg intravenously. Bottom = Dynamics of change (percent of initial level) in the area of the A response (- o -) and the C response (- ▲ -). x axis = Time in minutes. Right = Same after 6 mg/kg nonachlazine intravenously.

Thus, nonachlazine has a marked effect on the central control of circulation. This effect is evidently associated with activation of the inhibiting processes when average effective doses are used, and takes place more at the medulla oblongata level than in the system of the spinal reflex arches. It is important to note that data on the selective inhibition by this drug of reflex discharges initiated by impulses carrying "primary" pain signals agree with clinical observations that have found that a 1.5 percent solution per os will stop pain during angina pectoris within 2-5 minutes (29-31).

A 1-5 mg/kg dose of nonachlazine intravenously will sharply inhibit chemoreflexes from serotonin, but will not change the reflexes from sodium salicylate. Thus, nonachlazine can prevent some, but not all, chemoreflexes improving the tone of the parasympathetic nervous system and reducing the sympathetic influence on the heart.

Ethmozin--2-carbethoxyamino-10-(3-morpholylpropionyl)-phenothiazine hydrochloride--was derived from broad research in the 10-aminopropionyl derivatives of phenothiazine that demonstrated that the injection of the urethane groups into the phenothiazine molecule intensifies the antiarrhythmic properties of the compounds. Similarities do appear upon comparison of the spectra of pharmacological effects of ethmozin and nonachlazine. The antianginal properties of nonachlazine predominate, but it has an inherent antiarrhythmic effect as well. Ethmozin, on the other hand, because it is a typical antiarrhythmic, has not lost its capacity to improve the blood supply to the heart. The nature and mechanisms of the antiarrhythmic effect of ethmozin have been dealt with in detail in a number of articles (26,32-39). The following data resulted from study of the effect of ethmozin on the central process of sympathetic regulation of the heart. Ethmozin (1-3 mg/kg intravenously) increases the intensity of the reflex A response, greatly reducing discharges initiated by stimulation of the afferent C fibers (figure 3A). At the same time, there is a sharp intensification in the inhibiting component of the A response. The effect lasts for 20-30 minutes. Larger doses of ethmozin (up to 5 mg/kg) result in a sharp inhibition of tonic activity and reflex responses to stimulation of the A as well as C fibers (figure 3B).

Thus, 1-3 mg/kg ethmozin has a selective activating effect on reflex response from the high-threshold part of the afferent A fibers, the result of which is a substantial increase in the postactivation inhibition of tonic activity. It is possible that the result of this process is a reduction in the intensity of the C response. The basis for this statement is the observation made by Khaiutin et al. (14) pointing to the fact that convergence of late A and C responses takes place in the afferent part of the reflex arc at the level of the dorsal horn.

The effect of ethmozin on the spontaneous and reflex activity in the sympathetic nerves under reserpine treatment, as well as the blockage of the central adrenergic structures, was investigated for the purposes of studying the neurochemical mechanisms responsible for the initiation of the effects just described. It was established that neither reserpine treatment nor blockage of the β-adrenergic structures was able to eliminate the ethmozin effects described (figure 3C). There is no clear dependence of these effects on the influence of ethmozin on the central adrenergic process.

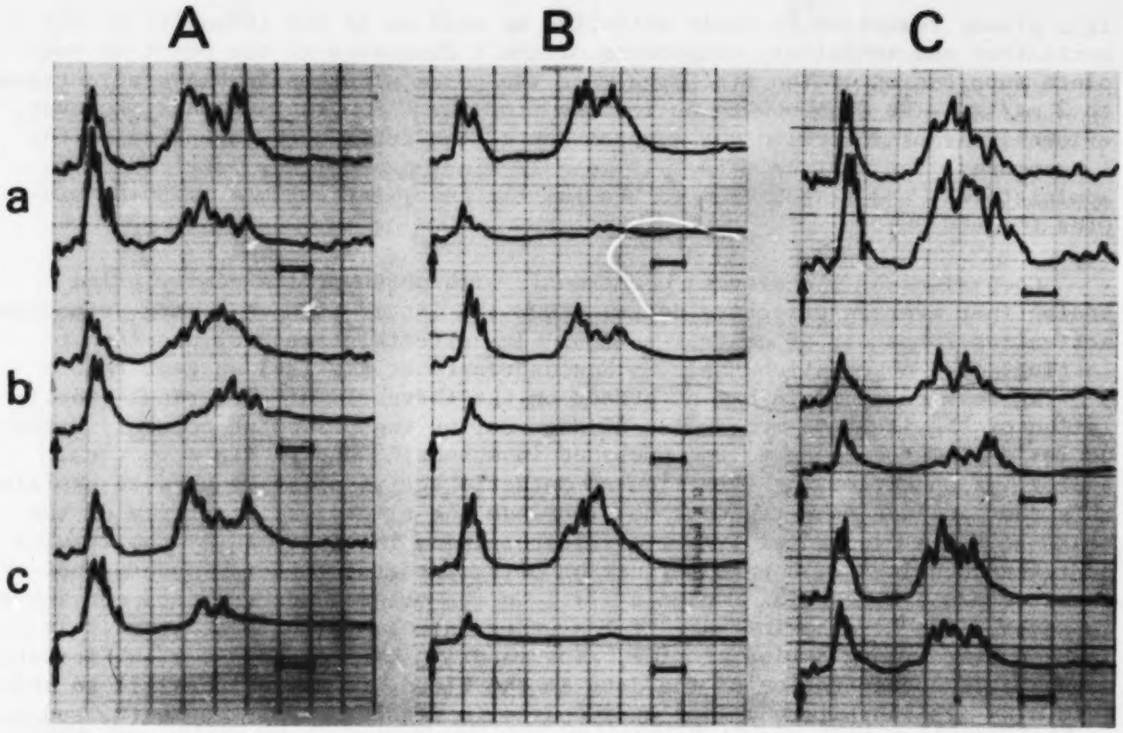

FIGURE 3. Effect of ethmozin and of its diethylamine analog (ethmozin DAA) on the reflex response in the inferior cardiac nerve upon stimulation of the afferent A and C fibers of the tibial nerve (averaged responses). For each pair of recordings, the upper curve is prior to injection of the drug, and the lower curve is 10 minutes after intravenous injection of the drug. Time tick = 1 sec. A: Ethmozin 1 mg/kg. B: Ethmozin 5 mg/kg. C: Ethmozin DAA 0.5 mg/kg. a = Effect of drugs on intact anesthetized cats. b = Effect of drugs 3 hours after 1 mg/kg (rausedil - 0.25 percent) reserpine, intravenously. c = Effect of drug 15 minutes after 3 mg/kg propranolol, intravenously.

A separate series of experiments was conducted to compare the effect of ethmozin with that of its diethylamine analog (ethmozin DAA), as well as with the effects of other antiarrhythmic drugs--lidocaine and mexiletine. It has already been established that ethmozin DAA has a much more intense and lasting antiarrhythmic effect than ethmozin, evidently associated with the well-defined and lasting inhibition of the fast inward sodium current (40). These data can be used in the future to search for effective antiarrhythmic agents among the acyl phenothiazines, structural analogs of ethmozin.

It has been determined that ethmozin DAA will, in effective antiarrhythmic doses (0.5-1 mg/kg), increase tonic activity and bring about a marked increase in the activating component of the A discharge. However, there is no reduction in the reflex response to an impulse from the C fibers, but rather something of an increase. The effect lasts for 40-50 minutes. There is a steady reduction in tonic activity, as well as in the intensity of the excitatory and inhibitory components of the A discharge to the point of complete suppression of the discharge when the doses of ethmozin DAA are increased to 2 mg/kg. The response to an impulse from the C fibers increases, however, evidently associated with the suppression of the inhibitory component of the A response. The maximum effect is seen 15 minutes after the latter dose is administered, and restoration to the initial level takes place in 50-60 minutes (figure 3C).

Comparison of the effects of ethmozin with those of its diethylamine analog (per average effective doses) shows the latter to have a more pronounced activating component of central action. Its effects, moreover, are longer lasting. The observations made by Rozenshtraukh et al. (34) suggest that a 3 mg/kg dose of ethmozin has no effect on the threshold of electrical fibrillation of the ischemic myocardium of dogs during the period of coronary artery occlusion. Our data as to the presence in ethmozin, and in its structural analog, of an activating effect on sympathetic activity can be used to explain this fact because ethmozin does not increase the electrical stability of the heart in acute myocardial ischemia. This opinion is confirmed by the results of research pointing to intensification of sympathetic activity under conditions of acute myocardial ischemia (7). On the other hand, our data can serve to advance the hypothesis that, if the ethmozin dose is increased, or if it is infused over a long period of time, thus ensuring maintenance of a sufficiently high stable concentration of the drug in the blood, it may be possible to bring into play its inhibitory effect on sympathetic regulation of the heart, and this, in turn, could help reduce the sensitivity of the myocardium to fibrillation.

Lidocaine and mexiletine. We will not dwell on the pharmacological properties of these agents because they have been widely reported in the literature (38). In our experiments, lidocaine over a range of doses from 1 to 5 mg/kg caused increasing inhibition of spontaneous bioelectrical activity in the sympathetic nerves, as well as of reflex responses to impulses transmitted by the A and C groups of fibers, with increase in dose. Some selectivity of its action with respect to reflex responses to impulses transmitted by the C fibers was noted. Increase in the lidocaine dose resulted in a marked reduction in spontaneous activity, and in complete inhibition of reflex responses in the sympathetic nerves. This effect lasted 50-60 minutes.

The effect of mexiletine was very similar to that of lidocaine. A 2 mg/kg dose reduced tonic activity and the intensity of the reflex responses to impulses transmitted by the A and C fibers. It completely inhibited spontaneous and reflex activity in the sympathetic nerves, beginning with a 5 mg/kg dose. The effect of 2-5 mg/kg doses lasted 15-20 minutes (figure 4).

The action of both agents resembles the effect of morphine-like narcotic analgesics in terms of the nature of the effect of relatively small doses. However, morphine characteristically has a much more marked selective inhibition of reflex responses to impulses transmitted by the C fibers.

From studying the effect of antiarrhythmic agents on coronary chemoreflexes we established that neither ethmozin nor lidocaine have the effect on

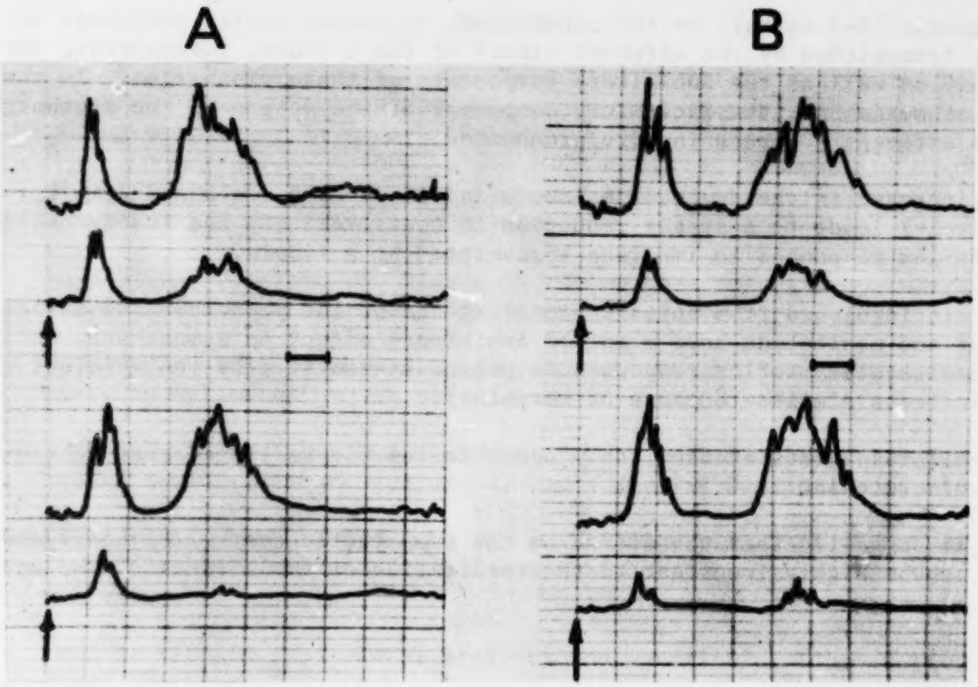

FIGURE 4. Effect of lidocaine (A) and mexiletine (B) on reflex response in the cardiac nerve upon stimulation of the afferent A and C fibers of the tibial nerve (averaged responses). The recordings are analogous to those in figure 3. A, top: Influence of 1 mg/kg lidocaine intravenously. Bottom: 5 mg/kg lidocaine. B, top: Influence of 2 mg/kg mexiletine intravenously. Bottom: 10 mg/kg mexiletine.

the reflexes demonstrated by serotonin, sodium salicylate, and α-naphthyl biguanide.

CONCLUSION

In this work, we have attempted to generalize data on the effect of certain antianginal (nitroglycerin, nonachlazine) and antiarrhythmic (ethmozin and its diethylamine analog, lidocaine, mexiletine) agents on sympathetic control of the heart. The effects of these agents with respect to reflexes from the heart under chemical stimuli (coronary chemoreflex) were also described.

Nitroglycerin (200-500 μg/kg) inhibits tonic activity and reflex responses in the sympathetic nerves of the heart, exerting its principal influence on discharge initiated by stimulation of the afferent A - δ fibers that transmit "primary" pain signals to the central nervous system. A new antianginal agent, nonachlazine, administered in average effective doses (5-6 mg/kg), has a very similar action.

Ethmozin (1-3 mg/kg), on the other hand, activates reflex responses to impulses transmitted by the afferent fibers of the A group, intensifying the excitatory as well as the inhibitory components of these discharges. In the case of ethmozin DAA, the excitatory component of the action on the discharges from the afferent A fibers is more pronounced.

An increase in the doses of ethmozin and its analog (to 5 and 2 mg/kg, respectively) leads to a steady reduction in tonic activity and the intensity of the reflex responses to impulses transmitted by A fibers.

As distinguished from antiarrhythmic agents of the phenothiazine series, lidocaine and mexiletine have a marked inhibitory effect on sympathetic activity, and also block reflex responses to pulses transmitted by the afferent C fibers (effects similar to those of morphine).

Of all the agents studied, only nonachlazine blocks the coronary chemoreflex to serotonin.

It is suggested that the effect on the sympathetic control of heart and vessels can be highly important to the realization of the effects of the agents studied.

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